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ERRATUM.

On page 65, Vol. XXVII, No. 1, January 1, 1918, the last two sentences of the second paragraph should read: *The difference between the total capacity and the middle capacity (all that can be breathed in after a half expiration) is called the "complementary air."*[2] *The difference between middle capacity and residual air (all that can be breathed out after a half expiration) is called the "reserve air."*

A NOTE ON THE PROGRESS OF CICATRIZATION OF WAR WOUNDS.

By THEODORE TUFFIER, M.D., and R. DESMARRES.

(From Auxiliary Hospital 75, Paris, France.)

Plate 6.

(Received for publication, March 26, 1917.)

The therapeutic effect of a physical or chemical agent on the cicatrization of wounds can be ascertained empirically from the rate of the process of cicatrization. To demonstrate this effect scientifically it is necessary first to determine exactly the normal evolution of a sterile wound,[1] that is the course of cicatrization in a given time; a unit of measure is thus obtained. When the normal progress of the cicatrization of a wound that is kept bacteriologically sterile is known, it becomes possible to study the changes taking place under physical or chemical influences.

This first question of a unit of measure was studied in Compiègne by du Noüy, under the direction of Dr. Carrel. We have worked according to du Noüy's formula, with the results here presented. All the work was done on wounded soldiers at Auxiliary Hospital 75.

To subject organic processes to a mathematical formula is always a hazardous procedure. However, we believe that by eliminating certain causes of error and adopting the controlled conditions of experimentation, results can be obtained which show the exact coincidence of mathematical forecasts with clinical evidence. This parallelism is constant and regular; if it becomes irregular, it is due to an error in therapeutic methods, thus furnishing a means of control of the care given to the patient.

The present work is the result of observations on twenty cases, in regard to the sterilization of the wounds as well as their rate of healing.

[1] By a sterile wound we mean bacterial or clinical sterilization; that is, with one or two cocci per field.

Cicatrization results from two processes, inodular contraction of connective tissue and epidermization. Contraction is in reality a mechanical action connected with the appearance of granulations on the surface of the wound.[2] We know from the work of du Noüy that a sterile surface wound heals in a pêriod of time which can almost invariably be predicted by the following simple calculation:[3]

If S_1 and S_2 represent the surface area of a wound before and after t days, then

$$S_1 = S_2 [1 - i (t - \sqrt{T})]$$

T representing the number of days elapsing since the first observation and i a constant characterizing each wound. This constant i, or the index, can be determined by means of the first two observations made, for example, at 4 days' interval.

i is expressed by the formula[4]

$$i = \frac{\frac{S_1 - S_2}{S_1}}{t + \sqrt{T}}$$

or for $t = T = 4$,

$$i = \frac{S_1 - S_2}{6S_1}$$

Du Noüy has shown that the index is clearly connected with the age of the patient and the initial surface of the wound; from a considerable number of observations, a chart[5] has been constructed which, on the assumption that the wound is sterile, makes it possible to determine the index *i a priori*, after only one measurement of the surface, when the age of the patient is known. The index varies approximately between 0.02 and 0.08.

The circumference of the wound or the epithelial edge can easily be traced on a sheet of sterilized cellophane; the surface is then measured in square centimeters with an Amsler planimeter. It is interesting also to observe at the same time the surface of the cicatrix; it can be seen almost invariably, during the days immediately preced-

[2] Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

[3] This information was kindly furnished by Captain Lecomte du Noüy, of Hospital 21, in Compiègne. For further detail see *J. Exp. Med.*, 1916, xxiv, 451, 461.

[4] du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 451.

[5] Text-fig. 1, du Noüy, *J. Exp. Med.*, 1916, xxiv, 463.

ing recovery, that the surface of the cicatrix broadens as if the contraction of tissue, having become unnecessary, ceased to occur.[2]

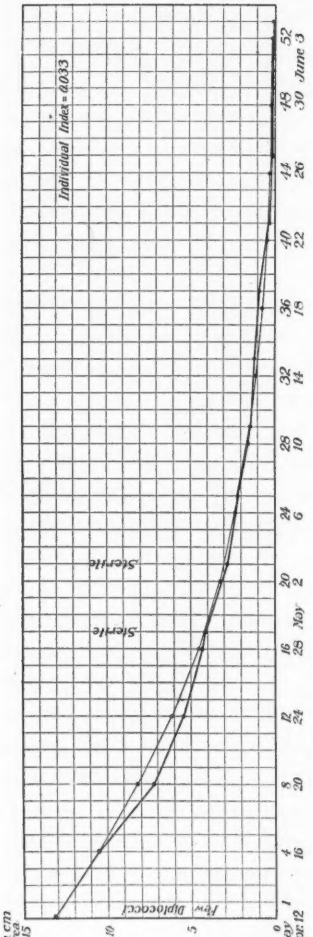
It is a simple matter to plot the calculated results or the direct observations in a curve with time as abscissæ and surface as ordinates. The curve resulting from the formula for $t = 4$, $T = 4, 8, 12, 16 \ldots .$ shows the normal course which the healing of a wound should follow. The observed surfaces give a second curve which follows the first closely unless there are irregularities to be explained by faulty dressings, reinfection, or illness of the patient. In the present experiments the theoretical curve is drawn with a light line, the curve from direct observation with a heavy line for the epithelial edge, and with a broken line for the cicatrix.

From the fact that the theoretical curve of cicatrization can be drawn in advance, with two measurements of the surface at 4 days' interval, it is seen that the date when cicatrization will be complete can be predicted, often long in advance (Text-figs. 1 and 2).

The technique is as follows: The wound is made clinically sterile by Carrel's method. We have then in the center a granulating surface (S, Text-fig. 3) surrounded by an epithelial border (L,L); beyond, at the periphery of the surface of the new epithelium, is a line of union of the normal skin and new epithelium (C,C). The wound closes by two processes: (1) contraction of the granulating tissue (inodular contraction); (2) epidermization of the granulating surface. These two processes have a relative efficacy for cicatrization, differing according to the wound.

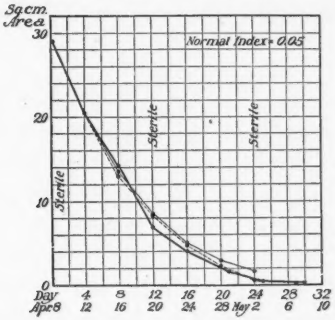
The course of cicatrization is most easily studied in a wound the site of which is not constantly in motion (as the bend of a joint), or one which is extendible and is well nourished, not a scalp wound or one due to loss of a large part of the skull.

The operator, having washed his hands with alcohol, applies to the wound, which has previously been dried with a sterile compress, a thin sheet of cellophane sterilized in the autoclave at 120°C. for 1 hour. In order to preserve the pliability of the cellophane during sterilization it is well to enclose it in a glass tube in the bottom of which is placed a tampon soaked in glycerol diluted one-fourth with water; the cellophane rests on a tampon of non-absorbent cotton, and another closes the tube.



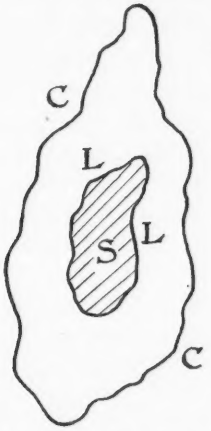
TEXT-FIG. 1. Experiment 1. Age 28 years. Superficial wound cicatrizing without incident, according to the formula.

Then with a dermographer's pencil, or, better, with pen and ink, as that allows a sharper and more exact line, a tracing is made of the



.....Curve corrected according to shape.

TEXT-FIG. 2. Experiment 2. Age 21 years. Long, narrow, superficial wound, cicatrizing without incident toward the date predicted. The corrected formula for this special kind of wound was employed in tracing the theoretical curve.[6]



TEXT-FIG. 3. Diagram of a wound, showing the granulating surface (*S*), surrounded by an epithelial border (*L*,*L*), and the line of union of the normal skin and new epithelium (*C*,*C*).

[6] For wounds of this sort du Noüy's corrected formula should be used:

$$S_n = S_{n-1}\left[1 - i\,(t + \sqrt{T})\right] - \frac{\sqrt{S_{n-1}}}{S_{n-1}}$$

See also du Noüy, *J. Exp. Med.*, 1917, xxv, 721.

epithelial edge and of the shape of the cicatrix. This is transferred to a sheet of ordinary paper, and on this are made the surface measurements with the planimeter.

Surface Wounds.

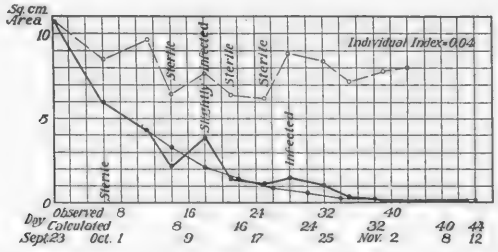
We have in this way studied a number of wounds treated with Dakin's solution by Carrel's method, keeping the bacterial flora regularly under observation both as to number and kind. Carrel's bacteriological curve was made for each wound.

Microscopic examinations, necessarily hasty, have no great value unless the results are positive, but the use of the curve sometimes points out a reinfection before it is observed, that is, before the microscope reveals it, as the observed curve deviates abruptly from the calculated curve,—which shows that cicatrization curves are not without value from the clinical point of view.

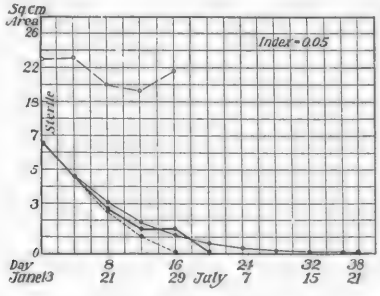
For wounds which are kept sterile the law holds, and cicatrization follows almost exactly the calculated curve (Text-figs. 1 and 2). If a wound previously sterile becomes accidentally infected and then becomes sterile again, the observed curve which separated from the calculated curve often rejoins it rapidly, and cicatrization is complete within a few days of the time predicted (Text-fig. 4). There seems to be a production of substances aiding cicatrization in the period preceding the infection. The substances are carried in the circulation but reinfection prevents their being used for epithelial cicatrization.

Cicatrization of certain wounds sometimes follows, at the beginning or later, a course more rapid than the calculated curve indicates. In such a case, if the wound is not a long, narrow one,[6] secondary ulcerations, in which we have never found bacteria, occur and restore the surface of the wound to what it would have been theoretically (Text-figs. 5 and 6). If infection entirely destroys epidermization, it is restored much more rapidly than the calculated curve indicates (Text-figs. 5 and 6). In Text-fig. 6 the observed curve oscillates about the calculated curve. It is difficult in this case to find a satisfactory explanation of this phenomenon; it can only be said that secondary ulcerations are related to the presence of bacteria in the deeper

parts of the wound. It is necessary in all such cases to know whether the bacteria are not retarding cicatrization. Certain cocci commonly observed allow normal cicatrization, but we have never found bacilli that allowed the same evolution of cicatrization.



TEXT-FIG. 4. Experiment 3. Surface wound of the right knee. The wound healed according to the law. Two infections in the course of cicatrization did not prevent the wound from healing in the calculated time.

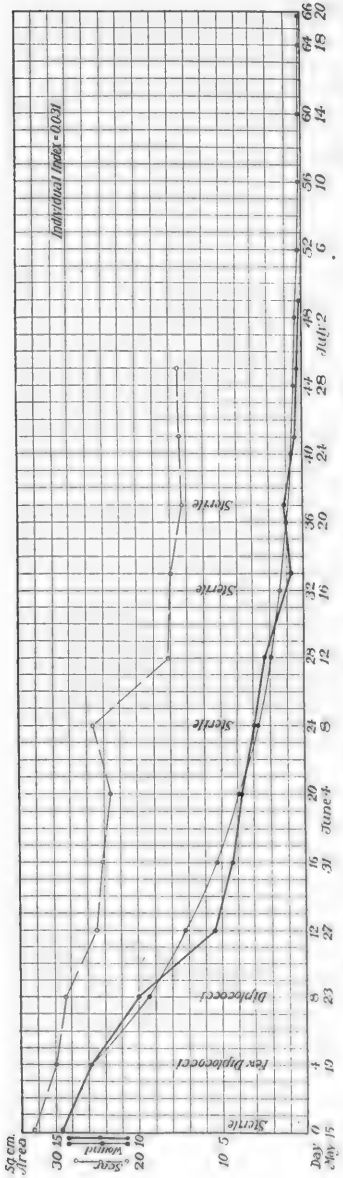


.....Curve corrected according to shape.

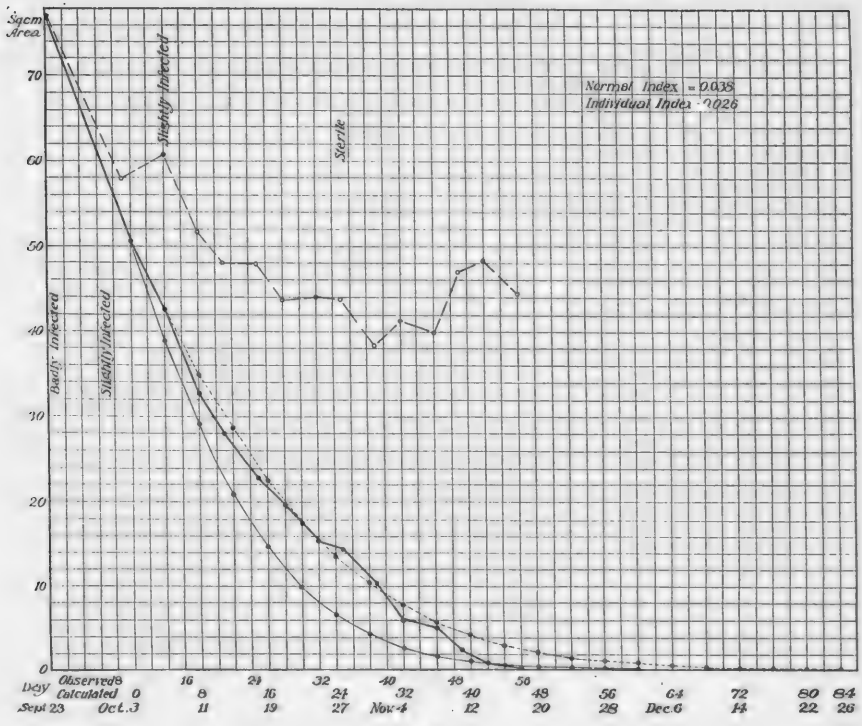
TEXT-FIG. 5. Experiment 4. Age 30 years. Superficial wound of the abdomen, in the iliac region. The wound was aseptic during the entire period of cicatrization. It healed at first more rapidly than the theoretical curve indicated, but more slowly than the curve from the corrected formula for long, narrow wounds.

From information subsequently received from the patient himself it was learned that the wound reopened and finally healed on July 19, as the curve had indicated.

Certain infective processes produce ossifying myositis or hospital gangrene, the first with an extension of the wound, which spreads out as a flower opens, the second with induration of tissue which will


Sq cm. Area
Scar Area
Wound
Individual Index = 0.09
Sterile
Sterile
Sterile
Sterile
Few Diplococci
Diplococci
Day 0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 66
May 15 19 23 27 31 June 4 8 12 16 20 24 28 July 2 6 10 14 18 20

TEXT-FIG. 6. Experiment 5. Age 31 years. Surface wound of the right leg. The observed curve oscillates about the calculated curve. When the real surface is smaller than the calculated surface, secondary ulcerations, in which no bacteria have been found, bring the observed surface back to a size very near the theoretical one. Hitherto no satisfactory explanation has been found for this phenomenon. See also Experiment 4.

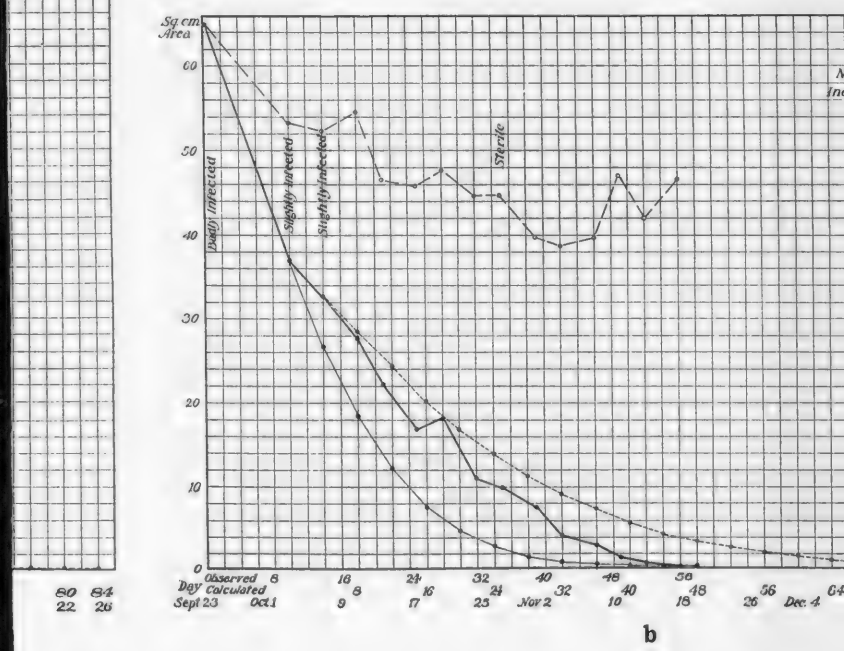

Sq cm Area
Badly Infected
Slightly Infected
Sterile
Normal Index = 0.038
Individual Index = 0.026
Day Observed 8 16 24 32 40 48 56 64 72 80 84
Day Calculated 0 8 16 24 32 40 48 56 64 72 80 84
Sept 23 Oct. 3 11 19 27 Nov. 4 12 20 28 Dec. 6 14 22 26

a

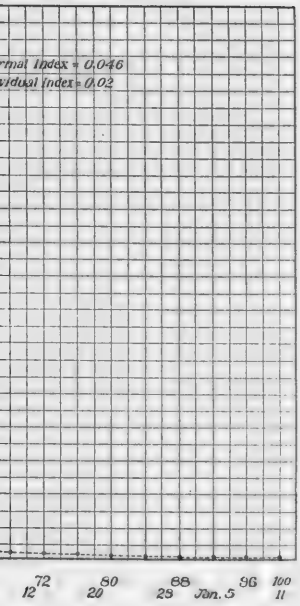
Calculated curve with normal index.

Calculated curve with individual index.

TEXT-FIG. 7, *a* and *b*. Experiment 6. Age 21 years. Two parallel wounds in the back, (*a*) upper wound, (show the effect produced by contraction of tissue; each wound alone would have cicatrized according to the calcu cm., and 21 years. The index calculated from the first two surface measurements gave a second theoretical between the two calculated curves, especially for the lower wound. From the time when contraction no longer the calculated curve.



n the back, (*a*) upper wound, (*b*) lower wound. The wounds were sterile during the peri
cicatrized according to the calculated curve, taking for the index the figures furnished by t
nts gave a second theoretical curve, drawn with a dotted line. The real curve of cica
me when contraction no longer occurred (the broken line toward the 46th day), cicatrizati



d of cicatrization. The curves
e chart,[5] for 50.35 and 36.85 sq.
rization remains almost entirely
n took place rapidly, overtaking

not contract. Thus it is clinically demonstrated that the nature of the infecting organism can influence the rapidity of cicatrization.

We have observed that if the mechanical contraction of tissue is hindered, by adhesions for instance, cicatrization occurs more slowly than is indicated by the calculated curve. Thus, cicatrization of two wounds near each other can be restricted by contraction in opposite directions. An example of this is Experiment 6 (Fig. 1, Text-fig. 7, *a* and *b*), two long parallel wounds in the dorsal region, generally sterile, restricting each other. Examination of the curves shows that the cicatrization of each wound if it had been alone would have followed the light line (index determined by the chart[5]); in reality it more nearly followed the dotted line (index determined from two actual measurements), at a slower rate. But in the last days before healing was complete, contraction of the tissues ceased (see the broken line), cicatrization went on much more rapidly, and the observed curve caught up with and even passed the calculated curve.

This experiment is related to a second theoretical cicatrization curve which we have frequently worked out. A continually infected wound can cicatrize according to the law provided, first, that the infection is within certain limits, that is that the number of bacteria is at most 14 to 15 per field; and second, that the infection is from cocci or diplococci, and that streptococci or bacilli are found only exceptionally. A theoretical cicatrization curve can be drawn from an index obtained from the two surfaces first measured. The observed curve will follow the calculated curve, and one should keep as close as possible to this.

Furthermore, a second theoretical curve may be made with the index determined by the chart,[5] from the initial surface of the wound and the age of the patient. This, which would be the cicatrization curve if the wound were sterile, is the normal curve, and we should try to follow it in treating the wound. The observed curve will lie practically between the two calculated curves (Text-fig. 8, *a*).

It seemed possible that the two curves could be of use when it is understood that the calculated index is not equal to the index determined by the chart, the two curves thus drawn constituting two limits between which the observed curve should continually remain.

Deep Wounds.

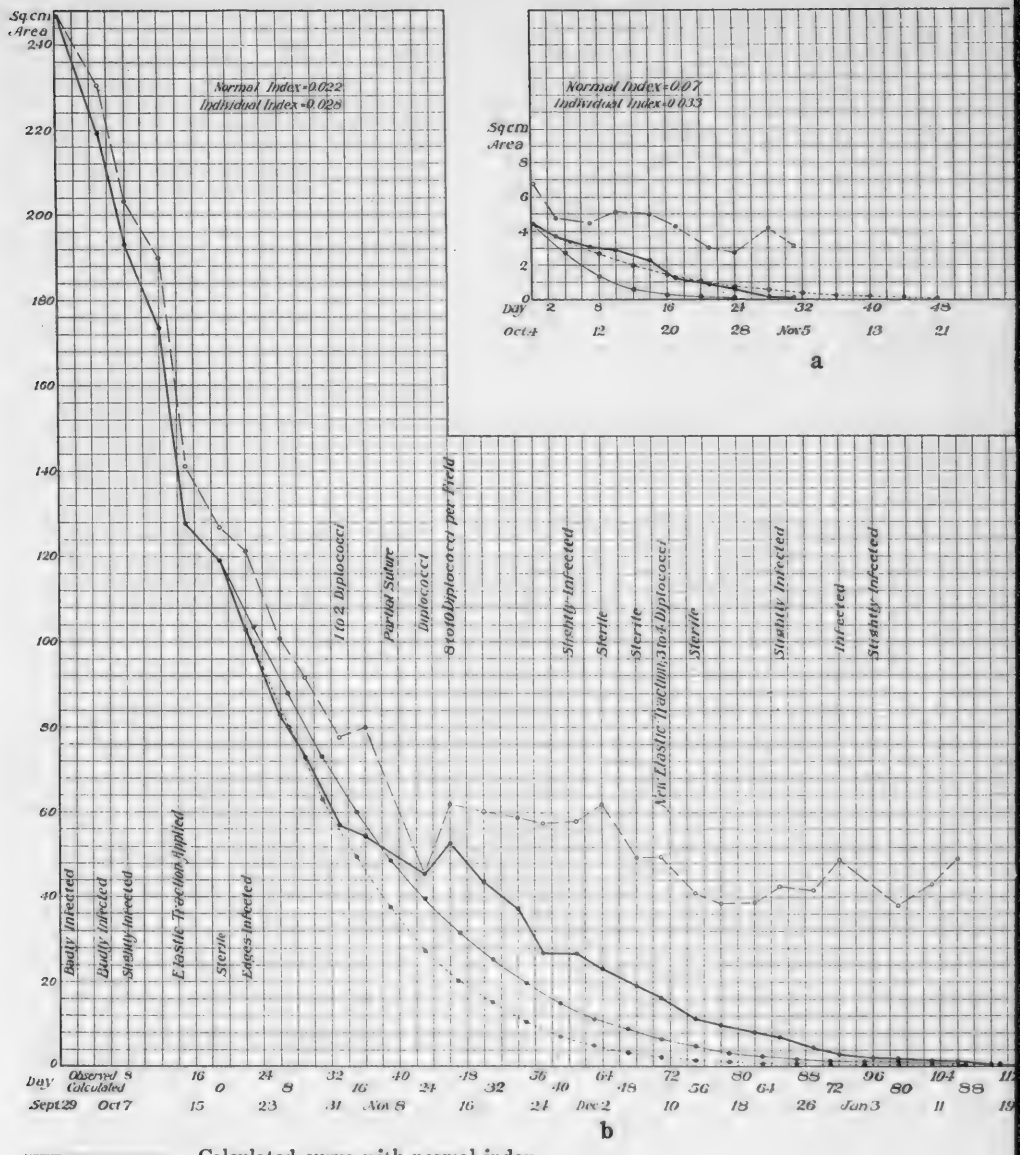
By an analogous procedure we have tried to follow the cicatrization of deep wounds. We first undertook to measure the volume of the wound by filling it with the fluid used for treating it, by means of a graduated pipette which could easily be sterilized. But the practical difficulty of placing the patient in a position in which the wound could be easily and thoroughly filled was so great, and the volume of the wound, which was often a cavity, was so variable, according to the patient's position, that we abandoned this procedure.

It seemed better to follow the indirect method of tracing on sterilized cellophane the successive outlines of the wound, as if we were dealing with a surface wound, and then to measure these areas.

By means of calculations identical with those which served for surface wounds, we have determined a theoretical cicatrization curve with an index calculated from the first two observed surfaces, or with an index determined from the chart. We have thus been able to show that a deep wound properly treated and sterilized, the edges of which are gradually drawn together at the proper time by adhesive plaster, with elastic strapping,[7] should cicatrize at least equally and often even more rapidly than a surface wound of the same contour (Text-figs. 9, *a* and *b* and 10, *a* and *b*). We have also observed that the gradual drawing together by means of the elastic traction is superior to the partial closure by suture intervening in the course of cicatrization (Text-fig. 8, *b*).

On October 13 the wound had been sterilized and the edges drawn together by an elastic traction; from that day cicatrization continued normally and steadily, and the observed curve lay between the two calculated curves (light line and dotted line). On November 7 the wound was partially sutured and decreased from 50 to 45 sq. cm., but presumably, from the general aspect of the curves, normal cicatrization would have decreased it to 35 or 36 sq. cm. After the operation the wound was reinfected and the surface extended to 52.75 sq. cm., decreasing again immediately, but at a much slower rate than was indi-

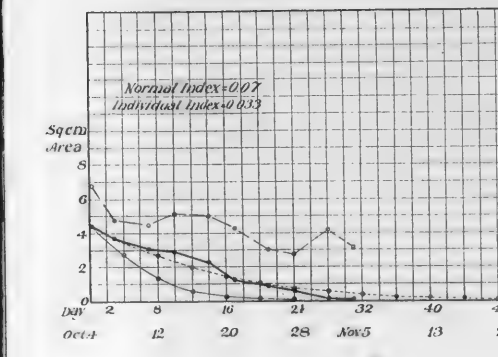
[7] Without the bandage a deep wound, at least one of small dimensions, seems to cicatrize according to a law analogous to that of the cicatrization of surface wounds, but more slowly.



——— Calculated curve with normal index.

..... Calculated curve with individual index.

TEXT-FIG. 8, *a* and *b*. Experiment 7. (*a*) Small wound. Example of cicatrization of a wound slightl infected. (*b*) Large deep wound. Example of a sterile wound whose edges have been drawn together by tion. An attempt at a partial suture was made, the action of which seems to have been injurious, if one co observed curve deviates from the calculated curve. A new elastic traction placed December 9 haste the observed curve tended to rejoin the calculated curves.



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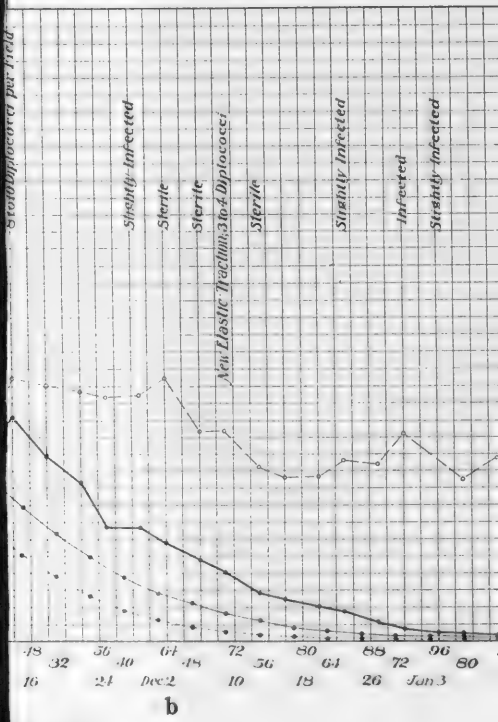

b

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Small wound. Example of cicatrization of a wou
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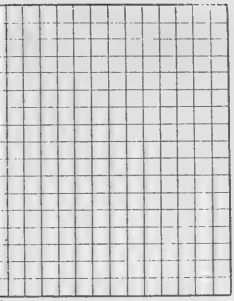

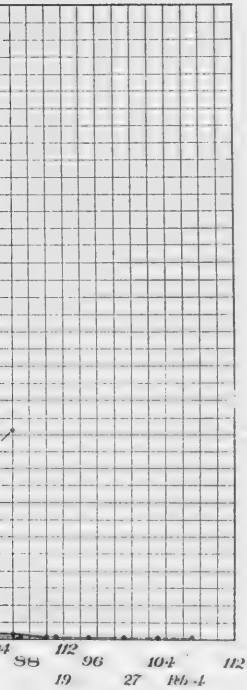

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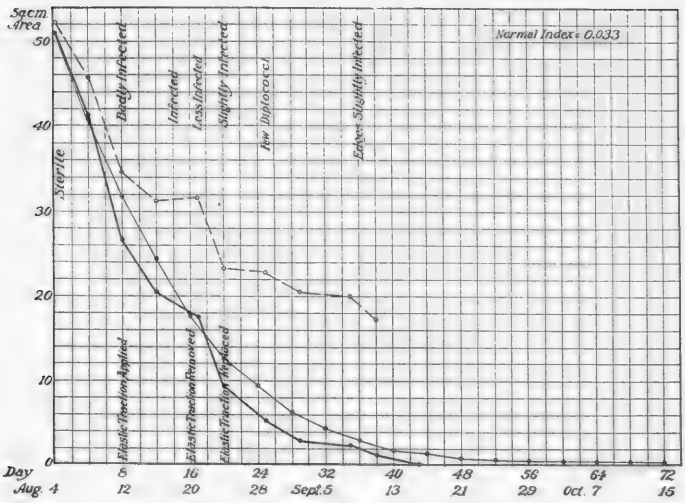
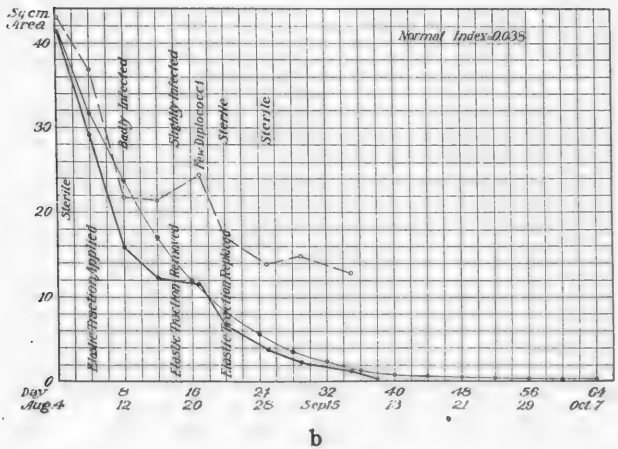


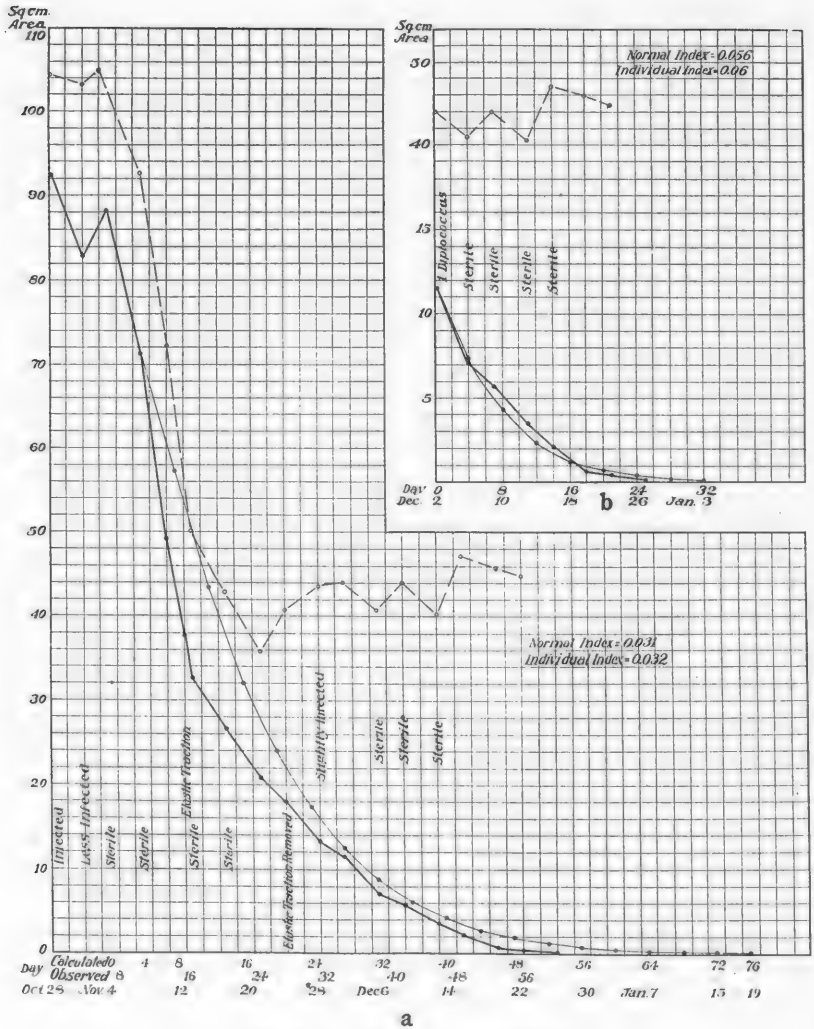
a



b

Calculated curve for superficial wound.

TEXT-FIG. 9, *a* and *b*. Experiment 8. Age 25 years. Deep hip wounds. The observed curves show the effect of the elastic traction on cicatrization, which took place rapidly in spite of the frequent presence of bacteria.



Calculated curve with normal index.

TEXT-FIG. 10, *a* and *b*. Experiment 9. Age 21 years. (*a*) Deep bullet wound in the lumbar region, treated with an elastic traction and cicatrizing more rapidly than a surface wound of the same contour. The smaller curve (*b*) was drawn after December 2, when the wound had become entirely superficial.

cated by the calculated curves, until the application of a second elastic traction again hastened the healing process. No conclusion can be drawn from this experiment, but it shows that in certain conditions in longitudinal wounds simply drawing the edges together gives rapid results.

SUMMARY.

Some hypothetical conclusions bearing on the evolution of cicatricial tissue can be suggested. The arterial circulation deposits in the wound chemical substances necessary for contraction of the wound and for epithelial proliferation. When the biôlogic process is not hindered by any special or severe bacterial infection this deposit is as regular as the circulation itself, and enables us to determine in advance the date of cicatrization. It even seems as though when the epidermization process is retarded by a slight infection the substances necessary for epidermization are stored up in the wound, and when the delay due to infection is removed the epithelium finds an accumulation of nutritive substances, and, so to speak, makes up the lost time.

Moreover, when an infection entirely or partially stops epidermization, we have observed (Experiments 3, 4, and 5) that after the infection has disappeared the progress of new epidermization is much more rapid than normally; it even passes the calculated curve. The infection apparently destroyed only the epithelium and left in the wound the chemical substances which activate epidermization.

The existence of these physical or chemical activating agents has been indicated again by two anatomical clinical facts. In treating a scalp wound in which there had been practically no epidermization for many months, we applied over the entire surface of the sterile wound dermo-epidermic grafts of fetal skin. After apparently taking, the grafts were absorbed and disappeared, but epidermization of the periphery of the wound, which hitherto had not progressed, took place abundantly, almost a hundred times as much as before.

We believe that by mathematical measurements we can solve the problem of the action of various organic fluids on the cicatrization of wounds.

EXPLANATION OF PLATE 6.

FIG. 1. Experiment 6. Age 21 years. Two parallel wounds in the back. Appearance of the wounds on October 6, 1916.

FIG. 1.

(Tuffier and Desmarres: Cicatrization of war wounds.)

STUDIES OF OXYGEN IN THE VENOUS BLOOD.

II. Studies of the Oxygen Unsaturation in the Venous Blood of a Group of Patients with Circulatory Disturbances.

By CHRISTEN LUNDSGAARD, M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 23, 1917.)

INTRODUCTION.

In a previous paper (1) a report was made of a series of determinations of the oxygen content of the venous blood from the vena mediana cubiti of twelve normal resting individuals. The difference between the oxygen in the venous blood and the total oxygen capacity of the hemoglobin (determined by Van Slyke's method (2) or Palmer's method (3)) was called the oxygen unsaturation of the venous blood. In this way the differences in hemoglobin concentration are eliminated.[1] It was found that the extent of oxygen unsaturation of the venous blood in normal individuals fell between 2.5 and 9 cc. per 100 cc. of blood. However, values above 8 volume per cent were only met with under special conditions; namely, when the blood was drawn in the morning immediately after the subject had been awakened from sleep. The blood was drawn from an arm vein without any stasis whatever, after the subject had rested for a half hour on a couch or bed. A sharp, not too pointed, needle was connected with a rubber tube to a glass pipette, 25 to 30 cm. long and $\frac{1}{2}$ cm. wide, which had a film of oxalate on the inside.[2] The blood (about 10 cc.) was sucked

[1] A determination of the oxygen in the venous blood without a simultaneous determination of the total oxygen capacity is just as incomplete as a determination of the urea in the blood or the nitrogen in the urine, without considering the intake.

[2] This was obtained by wetting the tube and rubber with a concentrated solution of oxalate and drying by an air current.

TABLE I.

Patients with Circulatory Disturbances Compensated at Rest.

Experimental.

Case No.	Determination No.	Age.	Bleeding.			Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen capacity (Van Slyke's method).	Oxygen unsaturation (a − v).	Pulse	Respirations.
						Sample 1.		Sample 2.		Average (v).						
			Date.	Hour.	Condition.*	Hour.	Result.	Hour.	Result.							
		yrs.	*1917*				*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*		
1	1	41	Feb. 3	11.00 a.m.	1 hr.	11.20	17.72	11.30	18.70	18.21	123	22.70	21.96 22.34	4.49	98	18
2	2	56	May 22	10.30 a.m.	In bed.	11.35	12.98	11.55	13.50	13.24	100	18.50		5.26	80	22
3	3	47	Feb. 12	4.00 p.m.	5 min.	4.15	15.30	4.25	15.14	15.22	100	18.50	18.74 18.00	3.28	84	24
	4		May 6	2.30 "	1 hr.	2.50	14.24	3.15	14.26	14.25			16.90 17.10	2.75	100	22
4	5	29	May 8	2.10 p.m.	In bed.	2.40	5.44	3.00	5.42	5.43	44.5	8.23		2.80	84	22
5	6	49	May 22	9.20 a.m.	In bed.	10.50	9.19	11.08	9.35	9.27	92	17.02		7.75	80	22
	7		June 21	11.00 "	" "	11.45	12.24	12.00	12.24	12.24	89.5	16.54		4.30	80	20
	8		" 27	9.30 "	¾ hr.	10.30	11.88		11.46	11.67	88.5	16.37		4.70		
	9		" 30	10.20 "	¾ "	10.35	10.89	10.55	10.93	10.91	88.5	16.37		5.46	80	20
6	10	22	June 26	9.50 a.m.	½ hr.	10.50	11.78	11.20	12.04	11.91	103	19.06		7.15	82	20
7	11	25	Apr. 18	12 n.	In bed.	12.40	13.52	12.55	13.60	13.56	111	20.53		6.97	76	24
8	12	37	Feb. 12	12 n.	In bed.	12.15	15.30			15.30	111	20.53		5.23	75	18
9	13	31	Feb. 17	3.00 p.m.	In bed.	3.50	17.15	4.15	16.90	17.03	114	21.10		4.07	67	26
	14		Mar. 8	10.00 a.m.	" "	11.10	14.62	11.40	14.42	14.52	106	19.60		5.08	90	22
10	15	57	June 27	12.30 p.m.	½ hr.	2.10	12.89	2.30	12.47	12.68	105	19.44		6.76	96	28
11	16	25	May 31	3.00 p.m.	¾ hr.	4.00	11.65			11.65	89	16.46		4.81	56	28
12	17	18	Feb. 28	10.30 a.m.	In bed.	10.55	6.72	11.10	6.75	6.74	71.4	13.20		6.46	80	22

Case No.	Determination No.	Oxygen unsaturation (a − v).	Diagnosis and clinical notes.
		vol. per cent	
1	1	4.49	Arteriosclerosis; hypertensio (blood pressure 160–96); hypertrophy of heart; shortness of breath on exertion.
2	2	5.26	Arteriosclerosis; hypertension; hypertrophy of heart (blood pressure 296–130); some moist râles in both lungs.
3	3	3.28	Chronic interstitial nephritis; hypertension (blood pressure 180–130); hypertrophy of heart; shortness of breath on exertion.
	4	2.75	
4	5	2.80	Chronic interstitial nephritis; chronic uremia; hypertension (blood pressure 195–133); hypertrophy of heart; edema; ascites; stasis in lungs (edema); urea retention in blood.
5	6	7.75	Chronic nephritis; hypertension; cardiac hypertrophy (he was never incompensated at rest. He had shortness of breath on slight exercise). May 22. A few râles at base of lungs; no dullness. Blood pressure 218–126.
	7	4.30	June 21. No râles. Blood pressure 214–108.
	8	4.70	" 27. Pains behind sternum. No râles. Blood pressure 220–112.
	9	5.46	" 30. No râles.
6	10	7.15	Aortic insufficiency; hypertrophy of heart (blood pressure 300–90).
7	11	6.97	Mitral stenosis; auricular fibrillation.
8	12	5.23	Mitral stenosis and insufficiency; auricular fibrillation.
9	13	4.07	Mitral insufficiency; auricular fibrillation.
	14	5.08	
10	15	6.76	Auricular fibrillation; chronic myocarditis.
11	16	4.81	Chronic myocarditis. Pulse regular.
12	17	6.46	Pericarditis; some râles at bases of lungs.

* Condition indicates the length of time the patient has been resting in bed before the drawing of the blood.

TABLE II.

Patients with Incompensated Heart Failure.

Experimental.

Case No.	Determination No.	Age.	Bleeding.			Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen capacity (Van Slyke's method).	Oxygen unsaturation (a − v).	Pulse.	Respirations.
			Date.	Hour.	Condition.	Sample 1.		Sample 2.		Average (v).						
						Hour.	Result.	Hour.	Result.							
		yrs.	*1917*				*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*		
13	18	30	Feb. 10	11.00 a.m.	1½ hr.	11.20	6.56	11.35	6.50	6.53	93	17.18	16.65 16.69	10.14		
	19		" 19	10.05 "	In bed.	10.30	6.67	1.45	6.58	6.63	100	18.50		11.87	62	22
14	20	57	Feb. 9	3.10 p.m.	In bed.	3.30	8.60	3.50	8.84	8.72			22.52 22.46	13.76	96	36
	21		" 19	10.00 a.m.	" "	10.10	5.93	10.50	5.79	5.86	112	20.71		14.85	86	32
	22		Apr. 27	2.40 p.m.	" "	2.45	12.39	3.10	12.55	12.47	120	22.20		9.73	86	32
	23		June 26	10.00 a.m.	" "	10.10	7.16	10.40	7.62	7.39	106	19.61		12.22	77	24
15	24	64	Feb. 21	11.10 a.m.	In bed.	11.20	12.12	11.30	12.05	12.07	119	22.00		9.91	36	20
	25		Mar. 24	12 n.	" "	2.30	7.68	2.50	7.66	7.67	117	21.65		13.98	28	20
	26		Apr. 21	11.00 a.m.	" "	2.10	10.48	2.30	10.46	10.47	110	20.36		9.89	32	24

Case No	Determination No.	Oxygen unsaturation (a — v).	Diagnosis and clinical notes.
		vol. per cent	
13	18	10.14	Mitral stenosis and insufficiency; auricular fibrillation; adherent pericardium.
	19	11.87	Râles at base of lungs; cyanosis of face; distention of veins in neck; enlargement of liver; ascites; low diuresis.
14	20	13.76	Mitral insufficiency; arteriosclerosis; hypertrophy of heart.
	21	14.85	Feb. 9. Moist râles in both lungs; severe cyanosis; enlargement of liver; severe edema of legs. " 19. Same condition.
	22	9.73	Apr. 27. Has improved. Very slight cyanosis; no edema; marked enlargement of liver. Still some râles in lungs.
	23	12.22	June 26. Slight edema of legs; slight cyanosis. Still râles at base of lungs.
15	24	9.91	Heart block; syphilis of heart. Feb. 21. Slight cyanosis of lips; slight jaundice of scleræ and skin; râles in lungs; no edema; great enlargement of liver.
	25	13.98	Mar. 24. Same condition.
	26	9.89	Apr. 21. " "

TABLE III.

A Patient with Incompensated Heart Failure. Oxygen Determined by Barcroft's Method.

Experimental.

Case No.	Determination No.	Age.	Bleeding. Date.	Bleeding. Condition.	Oxygen content of venous blood. Sample 1.	Sample 2.	Average (v).	Total oxygen capacity (Barcroft's method(a)).	Oxygen unsaturation (a − v).	Pulse.	Respirations.
		yrs.	*1916–17*		*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*		
	27	17	Oct. 25	In bed.	2.40		2.40	15.30	12.90		
	28		Nov. 20	" "	2.48		2.48	17.20	14.72	120	40
16	29		" 21	" "	3.36	1.36	2.36	17.56	15.20	122	40
	30		" 22	" "	2.40		2.40	17.56	15.16	124	44
	31		Jan. 12	" "	6.86		6.86	17.20	10.34	120	30

Clinical.

Case No.	Determination No.	Oxygen unsaturation (a − v).	Diagnosis and clinical notes.
		vol. per cent	
	27	12.90	Mitral and aortic insufficiency.
			Oct. 25, 1916. Moderate cyanosis; few râles at base of lungs.
	28	14.72	Nov. 20. Lungs clear.
16	29	15.20	" 21. Cyanosis.
	30	15.16	" 22. Enlargement of liver.
	31	10.34	Jan. 12, 1917. Less cyanosis; no râles; slight enlargement of liver.

up in the pipette, from which it was discharged into a cylinder, 2 cm. in diameter, below a layer (2 cm.) of mineral oil to prevent oxidation. The last (upper) 1 or 2 cc. of blood, a part of which had been oxidized, were put in a separate dish and used for a Palmer determination of the hemoglobin. Samples of blood for a Van Slyke determination were taken from the cylinder after careful stirring.[3]

Observations on Patients.

This paper is a report of a preliminary investigation on the oxygen unsaturation in patients with circulatory disturbances. Thirty-

[3] For further technical details see Paper I of this series (1).

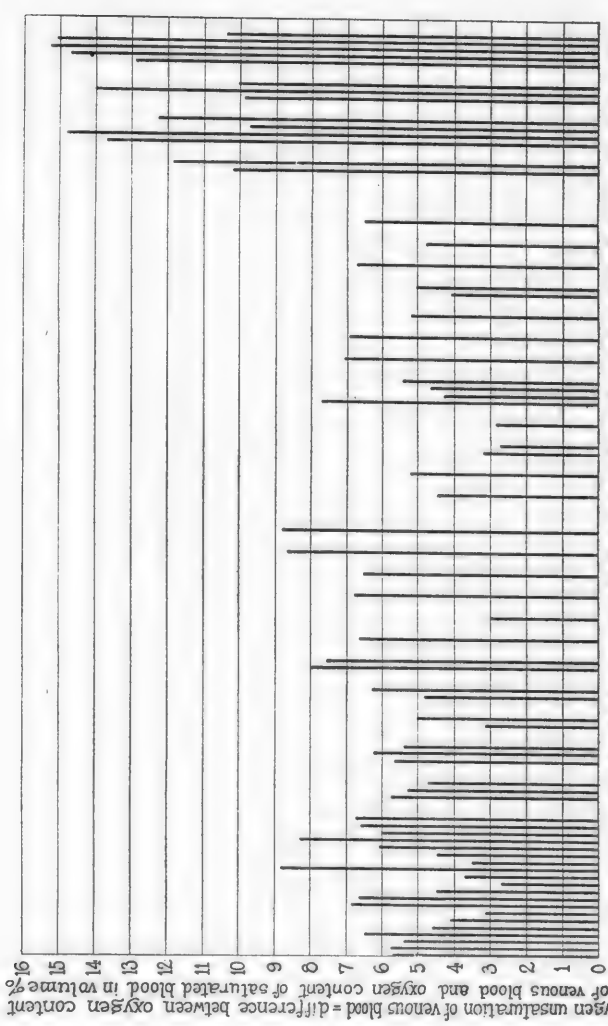
one determinations were made on sixteen patients with different kinds of circulatory disturbances. The procedure in preparing the patients and the technique in drawing the blood have been exactly as described in the first paper. Van Slyke's method (2) has been used for the determination of the oxygen in all but one case (No. 16 in Table III) where Barcroft's method has been used.

The patients have been divided into two groups according to the condition of the circulation. Results and clinical notes from patients with clinically compensated heart lesions have been tabulated in Table I. The data from the cases with incompensated circulatory conditions are collected in Tables II and III. As in Paper I, we have tabulated both the oxygen content of the venous blood samples and the extent of the oxygen unsaturation.

One case (No. 4, Table I) is particularly worth mentioning, because it shows the importance of using the oxygen unsaturation and not the absolute value of the venous oxygen alone in comparing the results from different individuals. This patient was brought to the hospital in a very sick condition. He was suffering from dyspnea and palpitation and had ascites and severe edema of the legs. The oxygen in his venous blood was 5.43 volume per cent, which was much lower than any figure obtained on normal individuals, and even lower than most of the figures obtained from patients with incompensated heart disturbances. The total oxygen-combining power of his blood was very low, however, 8.23 volume per cent, instead of the usual average 18.50 volume per cent. The oxygen unsaturation of his venous blood is therefore not only within normal limits but even rather low. The clinical diagnosis was chronic nephritis, chronic uremia, hypertension, hypertrophy of the heart, anemia, and urea retention. The edema was considered to be entirely of nephritic origin. Therefore, in respect to both the clinical picture and the findings in the blood, he is to be grouped in Table I, with patients free from incompensated circulatory disturbances. On the other hand, we may find a high figure for the venous oxygen combined with a high extent of unsaturation (No. 13, Table II) on account of a high content of hemoglobin.

Text-fig. 1 is a diagrammatic representation of the figures for the oxygen unsaturation in normal individuals[4] and in the patients re-

[4] Reported in Paper I (1).


oxygen unsaturation of venous blood = difference between oxygen content of venous blood and oxygen content of saturated blood in volume %
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Lu. St. F. St. B. Le. T. R. D. S. P. M.
Normal
1 2 3 4 5 6 7 8 9 10 11 12
Compensated Heart Conditions
13 14 15 16
Decompensated Heart Conditions

TEXT-FIG. 1. Oxygen unsaturation of venous blood in a group of normal subjects[4] and of patients reported in the present paper.

ported in the present paper. It will be seen that the upper limit for the unsaturation in normal individuals (9 volume per cent) is not exceeded by any figure obtained from patients belonging to the clinically compensated group. The figures are all distributed in the same haphazard manner over the area between 2.5 and 9 volume per cent.

The figures from the incompensated group show quite another picture. They are all above the upper normal limit, lying between 9.7 and 15.2 volume per cent.

No attempt has been made to subdivide the cases according to the anatomical or physiological form of the heart lesion. There may be a difference, but the cases and the number of determinations in each case are much too few to allow any such attempt. The only statement which can be made is that the oxygen unsaturation of the venous blood from patients with clinically compensated heart conditions has been found within the normal limits, whereas the oxygen unsaturation in some patients with incompensated heart lesions has been above the upper normal limit. It is probably not even justifiable to make this a general statement. It seems probable that patients with non-stationary conditions might fall outside this rule. We might suppose that a patient with an incompensated, but improving heart lesion could show normal figures. On the other hand, a patient with a heart lesion where incompensation is developing might possibly show increased oxygen unsaturation before the ordinary clinical symptoms had developed. If that should be true, the determination of the oxygen unsaturation would be of great clinical significance. Series of determinations on a single patient might throw light on this question.

DISCUSSION.

There is extensive clinical and experimental evidence for assuming that the condition of the heart has a certain influence on the extent of oxygen unsaturation of the venous blood. A previous investigation on the blood flow (minute volume) in patients with incompensated heart lesions (4) has shown a considerable decrease in the minute volume of the heart compared with that found in normal individuals

(5–14), and in most cases of compensated heart lesions[5] (4, 6, 11, 15). The figures obtained by Stewart (16) by determining the blood flow in the hands and feet point in the same direction. A retarded circulation, the rate of oxygen consumption not being decreased, may logically be assumed to result in a necessarily increased oxygen unsaturation of the average venous blood. The question is whether one is justified in assuming a retarded circulation from an increased oxygen unsaturation in the blood drawn from the vena mediana cubiti or from another superficial vein.

We know that other factors than the output from the heart may influence the extent of unsaturation in the blood from an arm vein. These factors are[6] (1) the possible unsaturation of the arterial blood; (2) variations in the metabolism of the tissues drained by the vein tapped as compared with the rest of the body; (3) variations in the rate of blood flow through the tissues drained compared with the rest of the body.

We are unfortunately, for the time being, only to a limited extent able to measure, control, or eliminate the influence of these factors.

(1) The dissociation curve for oxyhemoglobin shows that the blood must become nearly saturated (usually about 96 to 98 per cent) during the passage through the lungs, provided that all the blood flowing through comes into equilibrium with the alveolar air. This has been proved experimentally on animals by several investigators, and in

[5] Plesch (6) and Means and Newburgh (11) found normal figures for the blood flow in all their patients with compensated heart lesions, whereas Lundsgaard (4) found diminished minute volume not only in incompensated cases but in some patients with compensated heart conditions (mitral stenosis and auricular fibrillation), which agrees with Stewart's (16) observations for the local blood flow.

[6] One condition which might theoretically affect the oxygen saturation of the hemoglobin in arterial blood is incompensated acidosis, defined by Hasselbalch and Gammeltoft (17) and Van Slyke and Cullen (18), in which increased free carbonic acid causes an increase in the actual hydrogen ion concentration of the blood. As shown by Barcroft (19) this increase reduces the percentage oxygen saturation of hemoglobin under a given oxygen tension. Since, however, the differences thus caused in oxygen saturation become considerable only under reduced oxygen tension, it appears improbable that this factor, even in the infrequently occurring incompensated acidosis, exerts a significant effect on the degree of saturation of the arterial hemolgobin.

man it has been investigated by Hürter (20). By drawing blood from a radial artery and using Barcroft's method for determination of the oxygen, he found in four normal individuals a saturation of 94, 92, 99, and 100 per cent. In four patients with compensated heart lesions the saturation was 90, 100, 90, and 95 per cent. In one patient with incompensated aortic insufficiency and stasis bronchitis in the lungs the saturation was 81 per cent. In another patient with incompensation (Pick's disease) it was 92 per cent. A patient with patent ductus Botalli showed 88 per cent saturation. One with diffuse bronchitis showed 94 per cent, and another with phthisis, 88 per cent. Two patients with lobar pneumonia showed 81 and 79 per cent saturation. Barcroft[7] found a saturation of 94 per cent in a normal individual.

In several of my patients with compensated heart lesions moist râles have been heard at the base of the lungs. This circumstance does not seem to have influenced substantially the amount of oxygen in the venous blood. These râles are probably to a great extent just a proof that the air really does go down into the alveoli of the lowest part of the lungs. The whole problem is not sufficiently investigated to allow a decision concerning the influence of the involvement of the lungs in the individual case. We may confine ourselves to making a careful examination of the lungs in every case, hoping that special investigation of the unsaturation in patients with lung diseases or experimental studies will give us sufficient information.

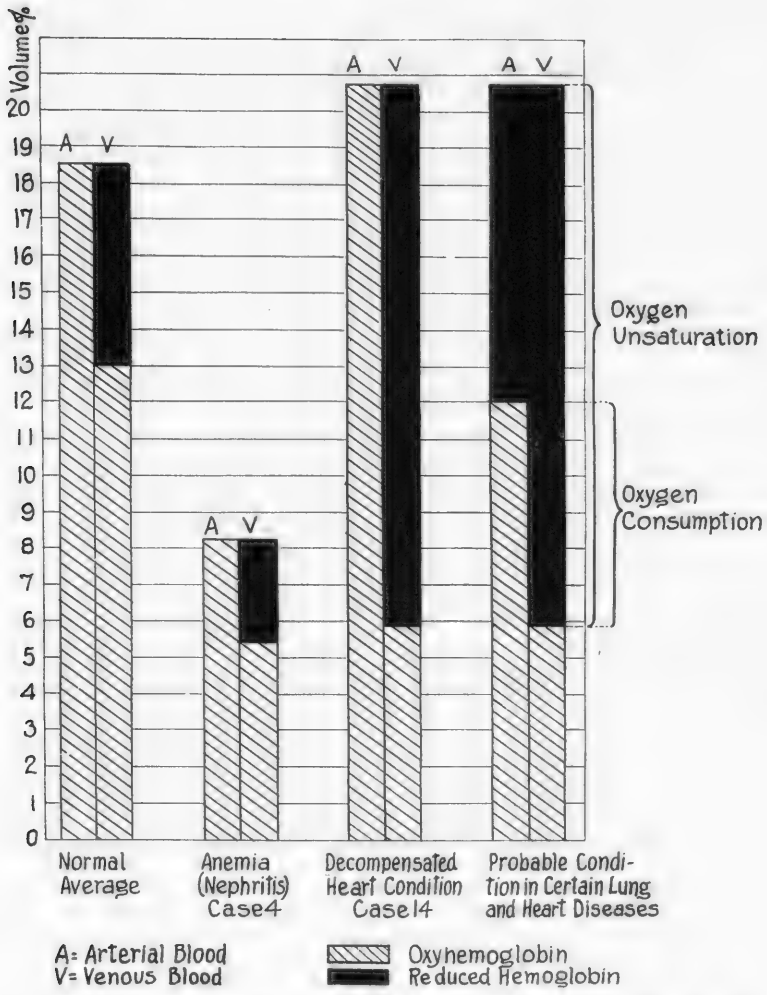
(2) The variations in the metabolism are presumably minimized by performing the experiments under definite conditions, such as muscular rest and digestive inactivity. The basal metabolism is a rather constant figure for the normal individual at rest and is, as shown by Lusk and his associates, chiefly dependent on the surface of the body. It has been shown by Peabody, Meyer, and Du Bois (21) that the metabolism in patients with compensated heart and cardiovascular lesions is within the normal limits. In patients with incompensated heart lesions, particularly in patients suffering from dyspnea, however, the metabolism was found materially increased, in some cases about 50 per cent. An increase in the metabolism necessarily means

[7] Barcroft (19), p. 177.

an increase in the consumption of oxygen. This, however, is not necessarily followed by an increase in the deoxidation of the oxyhemoglobin. That depends upon the output of the heart, which may or may not be changed. Lindhard (9), for instance, has shown that the increased consumption of oxygen during exercise only partially shows itself in the decreased oxygen in the venous blood; the increased blood flow compensates for a great part of the increased consumption. Lundsgaard (15) has shown that an increased oxygen consumption on account of exercise in two clinically compensated patients with heart block only to a very small degree could be compensated for by increasing blood flow. The reason for this was that it was impossible for the heart to increase the pulse rate and probably very difficult to increase the volume output per beat, which even in rest was about 150 cc. It is, therefore, probable that an increase in metabolism in a patient with a weak heart will increase the deoxidation of the venous blood, particularly in the venous blood coming from the heart muscle and the respiratory muscles. An increase in temperature may have a similar effect.

(3) About the only figures available on this point are those of Stewart (16). He has calculated from measurements of the heat given off by the hands and feet the rate of local blood flow and found that the flow in the right hand or foot is approximately equal to that in the left. Significant differences were encountered only when there was definite local cause, as aneurysms, diabetic gangrene, local edema, etc. It will probably be possible to throw light upon this question by simultaneously drawing blood from different veins and under different conditions, as tried in a number of cases by Means and Newburgh (11). Investigations on the local blood flow by Hewlett (22), using a modified Brodie method, have shown that there is a considerable temperature interval where the blood flow remains approximately constant when the surrounding temperature is changed.

The influence of the hemoglobin percentage, of the output from the heart, and of a possible unsaturation of the blood in the lungs is shown in diagram form in Text-fig. 2. The first double column represents the conditions in a person with normal hemoglobin, normal blood flow, and total saturation of the arterial blood. The next



TEXT-FIG. 2. Diagrams showing how the hemoglobin percentage, the output from the heart, and the saturation of the arterial blood influence the oxygen in the venous blood.

shows the findings in a patient with very low total oxygen capacity[8] but with normal circulation and full saturation in the lungs. A comparison of these two sets of figures shows the impossibility of using the zero as the base-line. The differences in hemoglobin must be accounted for. That is what we have done by using the term oxygen unsaturation, which is represented by the black area. The next two pairs of columns show how the extent of oxygen unsaturation can be affected in the same way by different causes. The third column represents the condition in a heart case (No. 14, Table II) when the total oxygen capacity was a little above normal; the saturation in the lungs was supposed to be normal. The cause of the increased oxygen unsaturation in this case was a slow circulation. The fourth column represents a hypothetical case with the same degree of oxygen unsaturation of the venous blood. However, the cause is ascribed to a considerable extent of unsaturation of the arterial blood. These two examples show the possible inaccuracy of using the word oxygen consumption in the same sense as oxygen unsaturation.

Oxygen Unsaturation Compared with Oxygen Consumption (Blood Flow).

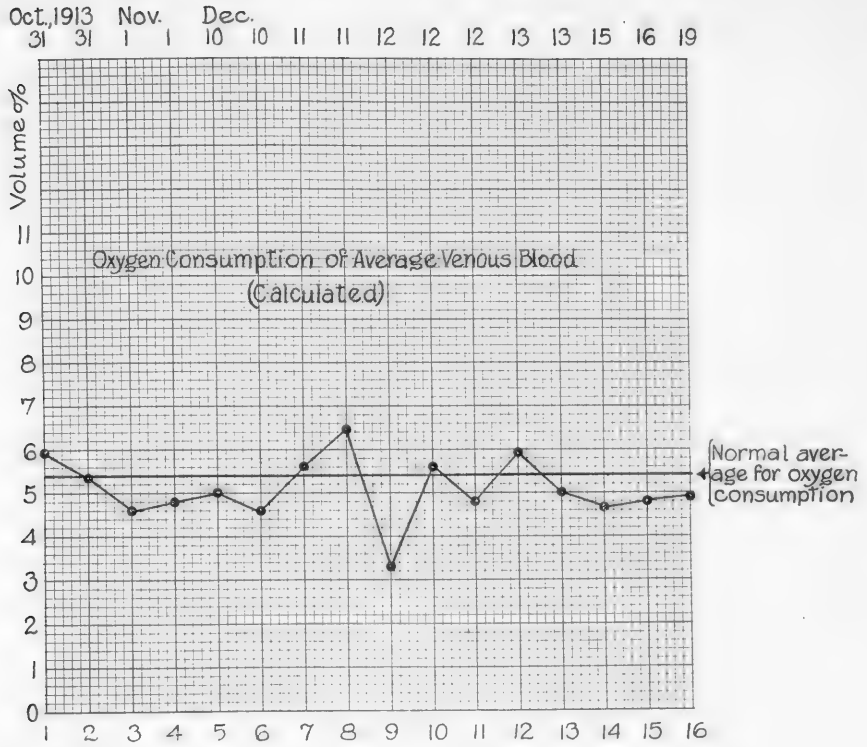
It is of interest for the interpretation of our results that the minute volume (output per minute from the heart) was determined 4 years ago in the Medical Clinic of the University of Copenhagen[9] on the same subject (the writer) on whom twenty determinations of the oxygen unsaturation of the venous blood have been done (Text-figs. 1 and 4).[10] From the figures indicating the values for the minute volume we can calculate the oxygen consumption of the average venous blood.[11]

[8] Compare Morawitz and Röhmer's observations (23).

[9] Lundsgaard (14), p. 397.

[10] See Table I, Paper I (1).

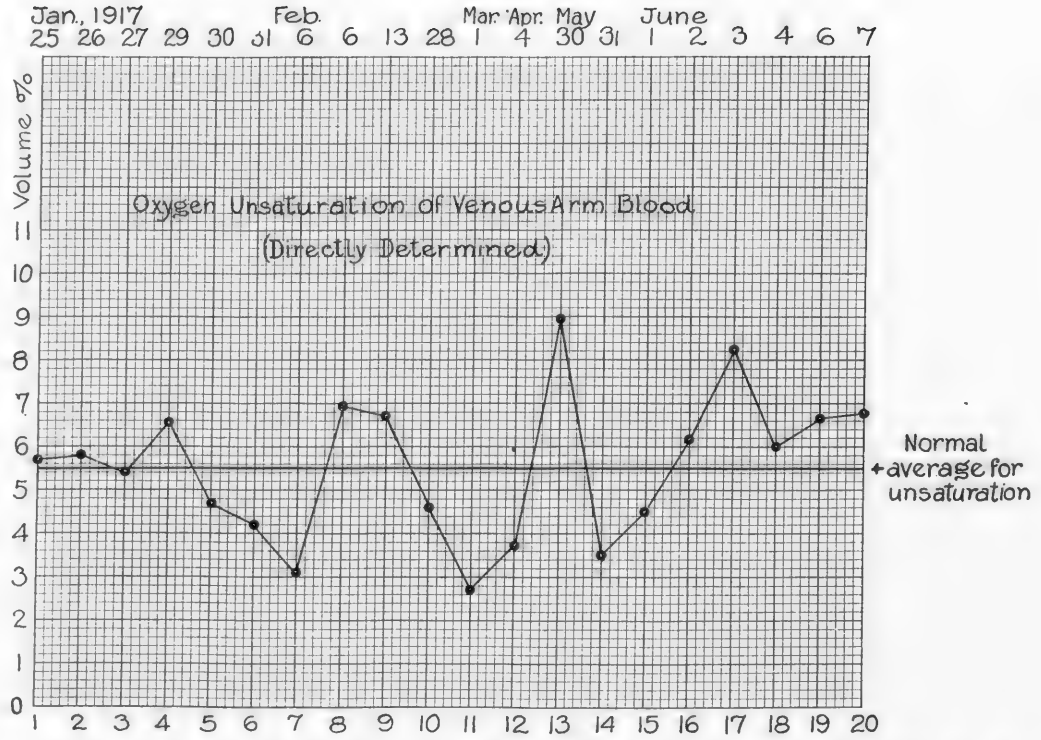
[11] The details of Krogh and Lindhard's method can be seen in the papers quoted in the bibliography, particularly in 8, 10, 13, and 14. The principles are the following: The subject is connected with an easily movable spirometer which contains a mixture of air and nitrous oxide. He mixes his lung air with the air in the spirometer during three to five respirations. He then stops breathing for a few seconds. Before and after the apneic period a sample of alveolar air is


Oct., 1913 Nov. Dec.
31 31 1 1 10 10 11 11 12 12 12 13 13 15 16 19
Volume %
0 1 2 3 4 5 6 7 8 9 10 11
Oxygen Consumption of Average Venous Blood
(Calculated)
Normal average for oxygen consumption
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

TEXT-FIG. 3. Diagram showing the oxygen consumption of a normal man calculated from sixteen determinations of the minute volume of the heart by Krogh and Lindhard's nitrous oxide method.[12]

drawn and analyzed. On account of absorption by the blood flowing through the lung capillaries the second sample will contain a smaller amount of nitrous oxide and of oxygen than the first. The percentage in the difference between the two samples multiplied by the volume of air in the lungs will indicate the amount of blood flowing through the lungs during the period between the two samples. The output of blood per minute from the heart (the minute volume) can then be calculated. Knowing the quantity of oxygen taken up by the blood during the same period we can calculate the amount of oxygen taken up by the body per 100 cc. of blood. When a correction is introduced for a possible abnormality in the percentage of hemoglobin this amount will indicate the oxygen consumption by the average blood. An extensive critical study of Krogh and Lindhard's method is published by Sonne (24). He expresses doubt about the reliability of the method on account of possible imperfect mixture of the lung air, not only in patients but in normal individuals as well. Krogh and Lindhard (25) have later on admitted that the difficulties shown by Sonne may exist.

[12] See Lundsgaard (14), p. 397, where the data for the blood flow can be obtained.


Jan., 1917
Feb.
Mar. Apr. May
June
25 26 27 29 30 31 6 6 13 28 1 4 30 31 1 2 3 4 6 7
Volume %
Oxygen Unsaturation of Venous Arm Blood
(Directly Determined)
0 1 2 3 4 5 6 7 8 9 10 11
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Normal
average for
unsaturation

TEXT-FIG. 4. Diagram showing the oxygen unsaturation of the venous arm blood from the same normal subject[4] as in Text-fig. 3 (see also Text-fig. 1).

Sixteen determinations of the blood flow were made at that time. The calculated figures for the oxygen consumption in these sixteen experiments are given in Text-fig. 3. The average oxygen consumption for a considerable number of blood flow experiments on normal people (5.4 volume per cent) is indicated.

In Text-fig. 4 are given the figures for the oxygen unsaturation of the venous arm blood of the same subject (the writer) as reported in the first paper of this series.[10] The average line in this diagram (5.5 volume per cent) is from thirty-eight determinations of the oxygen unsaturation on eleven normal people reported in Paper I and shown in Text-fig. 1 in this paper. It will be seen how closely the values for the oxygen unsaturation determined by Van Slyke's method agree with the values for the oxygen consumption calculated from the determinations of the blood flow with Krogh and Lindhard's method. The average figures for normal individuals are the same (5.5 and 5.4 volume per cent). The variations for the two series on the same person agree rather closely. The variations in the oxygen unsaturation are more extensive than the variations in the values for the average consumption; *i.e.*, for the blood flow. This is probably due to variations in the local blood flow in the arm from which the blood is drawn. It is worth mentioning that the subject on whom the determinations were done has a very labile circulatory system (respiratory arrhythmia, changeable pulse, dermographia).[13] The significance of this agreement is that the amount of oxygen lost by 100 cc. of blood in passing through the forearm is approximately the same as the average loss in passing through the other body tissues. In view, nevertheless, of the undoubted possibility that the disturbing factors discussed may influence the unsaturation, we are not justified at present in interpreting unsaturation figures in terms of minute volume of the heart. What we believe we can do, is to fix the limits of the oxygen unsatura-

[13] The conception that the extensive variations in the oxygen unsaturation in this particular case are principally due to vasomotor changes, is supported by an observation (Cohn and Lundsgaard, personal communication) on the relation between the brachial blood pressure and the blood pressure in the arteries of the finger. The tension in the digital arteries was found more variable than in other normal subjects and more variable than the blood pressure nearer the center.

tion in subjects with normal circulation and normal lungs and study the variations observed in carefully controlled patients with symptoms of abnormal circulation and in patients with respiratory abnormalities. From the data thus obtained we may empirically standardize the figures for the oxygen unsaturation, learn the pathological conditions that affect it, and thus add it to the armamentarium that assists the clinician in accurate diagnosis. The empirical evolution of blood pressure measurement, made possible by accumulation of many determinations on clinically controlled patients, has shown how a quantitatively measurable factor, even though imperfectly explained physiologically, may prove to be of value in clinical medicine.

SUMMARY.

1. Thirty-one determinations of the total oxygen-combining power and the oxygen in the venous blood from vena mediana cubiti of sixteen resting patients are reported.

2. The difference between the total oxygen capacity of the hemoglobin and the oxygen in the venous blood, the oxygen unsaturation, is calculated.

3. In twelve patients with compensated heart lesions the unsaturation was found within normal limits, between 2.5 and 8 volume per cent.

4. In four patients with incompensated heart disease the values for the unsaturation were all above the normal limit, from 9.7 to 15.2 volume per cent.

5. A general discussion of the problem of interpreting the results is given.

6. A comparison is drawn between the oxygen consumption calculated from direct determination of the blood flow on a normal subject (the writer) and the oxygen unsaturation determined 4 years later on the same subject. A close agreement between the two series of values exists.

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STUDIES OF OXYGEN IN THE VENOUS BLOOD.

III. Determinations on Five Patients with Compensated Circulatory Disturbances.

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INTRODUCTION.

In two previous papers on the same subject (1, 2) the technique of drawing blood from the arm vein and a series of determinations on twelve normal individuals and sixteen patients with circulatory disturbances have been reported.

In the discussion of the interpretation of the results, it was pointed out that variations in the oxygen unsaturation of the blood from an arm vein might be caused by several factors other than the output from the heart, which we were able to control or eliminate only to a limited degree. These factors were: (1) the degree of oxygen saturation of the arterial blood; (2) the oxygen consumed by the metabolism of the part of the body drained by the vein tapped; (3) changes in the local blood flow on account of vasodilatations or constrictions either in the organ (the arm) from which the blood is drawn or in other parts.

In animal experiments the influence of these factors might be studied separately. In clinical medicine, however, we have to deal with a given condition where several factors usually act together. We are, therefore, compelled to compare our findings with the clinical observations and postpone the conclusions until sufficiently extensive material has been collected.

The purpose of these investigations is to study the variations in the oxygen unsaturation of the venous blood in patients with symptoms of circulatory disturbances; that is, in patients in whom it is of importance to find out whether the blood flow is changed or not.

The preliminary determinations reported in Paper II gave promising results. Continuous determinations on patients over a longer period have therefore been taken up. Ten patients have been studied, five with compensated[1] and five with incompensated circulatory disturbances. This paper is a report of the findings in the compensated group.

Before reporting the clinical notes and the figures for the oxygen unsaturation in these five patients, one should recall that the limits for the oxygen unsaturation in normal individuals were 9 and 2.5 volume per cent. However, values between 8 and 9 volume per cent were found in only four determinations and under special circumstances—a few seconds after awakening from a night's sleep, a condition which, as a rule, we shall be unable to reproduce in patients. Considering that all the values for the oxygen unsaturation in twelve patients with compensated circulatory disturbances (2) fell between 8 and 2.5 volume per cent, it seems correct, at least for the time being, to consider 8 volume per cent the upper normal limit. The lower limit is 2.5 volume per cent, the average 5.5. In the diagrams of the oxygen unsaturation lines have been drawn to indicate the normal limits.

CASES.

Case 1 (Table I, Text-Fig. 1).—V. G., male, gardner; age 58 years.

Diagnosis.—Aortic insufficiency; arteriosclerosis; hypertension.

Previous History, Symptoms, etc.—Dyspnea after slight exertion, for 1 year. He has never had syphilis or rheumatic fever, but for years he has suffered from stiffness of the back and has now and then had slight joint pains.

Physical Examination.—No cyanosis; no swelling of superficial glands; lungs clear; heart dullness increased considerably to the left and downwards; x-ray shows increase to the left and downwards; no thrill. At the fourth rib on the left side is heard a distinct diastolic murmur replacing the second sound, also heard at apex; liver not felt; no ascites; no edema. In the urine was found a trace of albumin; no casts. Blood pressure 150–170. Wassermann reaction negative. Temperature normal.

He was admitted to the hospital for diagnosis, confined to bed for the first 3 days, and after that time allowed to get out of bed. No special treatment. He felt better when discharged; dyspnea less; physical signs unchanged.

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[1] Patient 4 in the present paper had symptoms of heart failure. During the course of the disease the symptoms were interpreted as not circulatory.

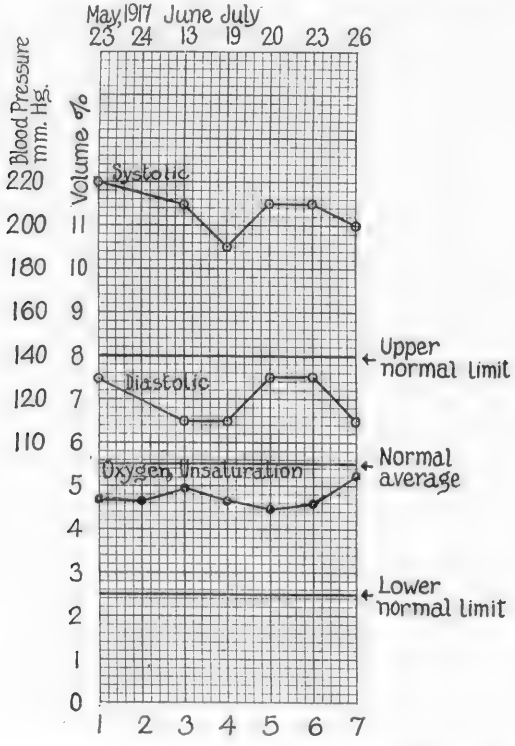
TABLE I.

Case. 1. Aortic Insufficiency; Arteriosclerosis; Hypertension; Hypertrophy of Left Side of Heart.

Determination No.	Bleeding.				Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a – v).	Pulse.	Respirations.
	Date.	Hour.	Arm.	Condition.*	Sample 1.		Sample 2.		Average (v).					
					Hour.	Result.	Hour.	Result.						
	1917					*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	May 23	1.00 p.m.	Left.	¾ hr.	2.30	13.90	2.50	13.82	13.86	100	18.50	4.64	72	24
2	" 24	1.00 "	Right.	¾ "	3.00	14.29	3.10	13.64	13.97		(18.50)	4.53	76	20
3	June 13	11.50 a.m.	"	¾ "	12.20	13.55	12.50	13.46	13.51	100	18.50	4.99	66	24
4	July 19	11.00 "	Left.	1½ hrs.	11.20	14.01	12.00	14.89	14.45	103	19.06	4.61		22
5	" 20	11.50 "	"	18 "	1.10	14.68	1.30	14.68	14.68	103	19.06	4.48	66	22
6	" 23	9.30 "	Left and right.	12 "	10.20	14.60	10.50	14.32	14.46	103	19.06	4.60	66	22
7	" 26	9.40 "	Right.	½ hr.	10.20	13.82	10.40	13.62	13.72	103	19.06	5.34	72	24

* Condition indicates the length of time the patient has been resting in bed before the drawing of the blood.

This patient, who suffered from a valvular heart lesion which is fully compensated at rest, shows almost constant values for the oxygen unsaturation from day to day. Furthermore the values follow the normal average. He was a patient with a quiet temper, with very slowly reacting vasomotors and with a steady, regular pulse.



TEXT-FIG. 1. Oxygen unsaturation of venous arm blood. Case of aortic insufficiency; arteriosclerosis; hypertension.

Case 2 (*Table II*, *Text-Fig. 2*).—T., male, steward; age 52 years.

Diagnosis.—Hypertension arterialis; slight arteriosclerosis; chronic alcoholism; cirrhosis of the liver (?); lues.

Previous History, Symptoms, etc.—Shortness of breath on exertion, for 4 years.

Physical Examination.—Rather fat man; lungs clear; no cyanosis; no prominent

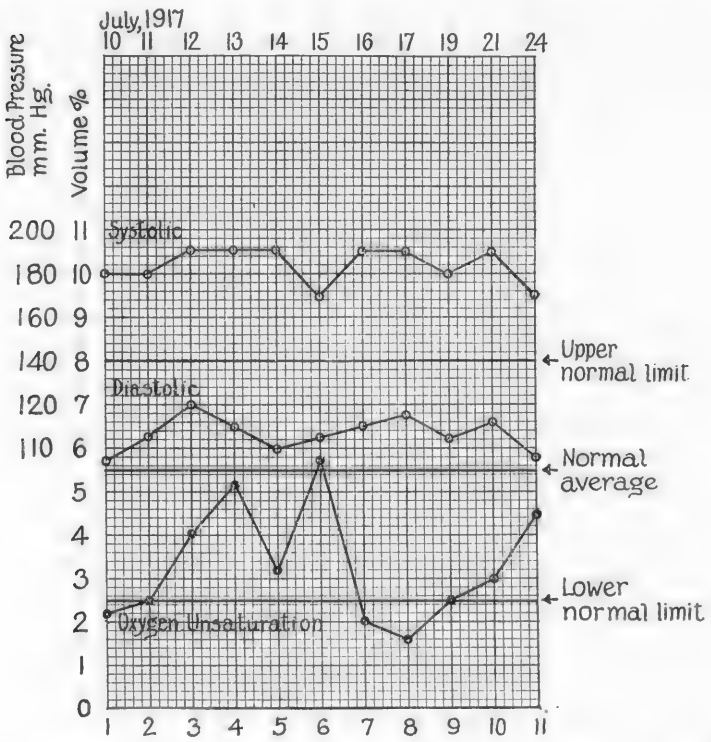
TABLE II.

Case 2. Arterial Hypertensions; Slight Arteriosclerosis; Cirrhosis of Liver; Lues; Chronic Alcoholism.

Determination No.	Bleeding.				Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a – v).	Pulse.	Respirations.
	Date.	Hour.	Arm.	Condition.	Sample 1.		Sample 2.		Average (v).					
					Hour.	Result.	Hour.	Result.						
	1917					*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	July 10	9.30 a.m.	Left.	In bed.	10.30	17.02	10.50	16.80	16.91	103	19.06	2.16	73	20
2	" 11	9.30 "	"	" "	4.10	16.62	4.40	16.62	16.62	103	19.06	2.44	84	24
3	" 12	9.30 "	Right.	" "	10.10	15.05	10.50	15.05	15.05	103	19.06	4.01	87	20
4	" 13	9.30 "	"	" "	11.10	13.83	3.40	13.99	13.91	103	19.06	5.15	92	28
5	" 14	9.30 "	"	12 hrs.	2.10	14.60	2.20	14.80	14.70	97*	17.92	3.22	92	22
6	" 15	12.20 p.m.	"	12 "	12.40	12.07	12.55	12.81	12.44	98*	18.13	5.69	91	22
7	" 16	10.30 a.m.	"	12 "	10.40	16.85	10.53	16.85	16.85	102	18.82	1.97	90	24
8	" 17	9.20 "	"	12 "	10.20	17.50	11.00	17.40	17.45	103	19.06	1.51	92	28
9	" 19	9.30 "	Left.	12 "	10.00	15.97	10.20	15.97	15.97	100	18.50	2.53	98	22
10	" 21	9.30 "	"	12 "	9.40	15.82	10.10	15.18	15.50	100	18.50	3.00	96	24
11	" 24	9.30 "	Right.	1 hr.	10.00	13.25	10.15	13.25	13.25	96	17.74	4.49		26

* Checked three times.

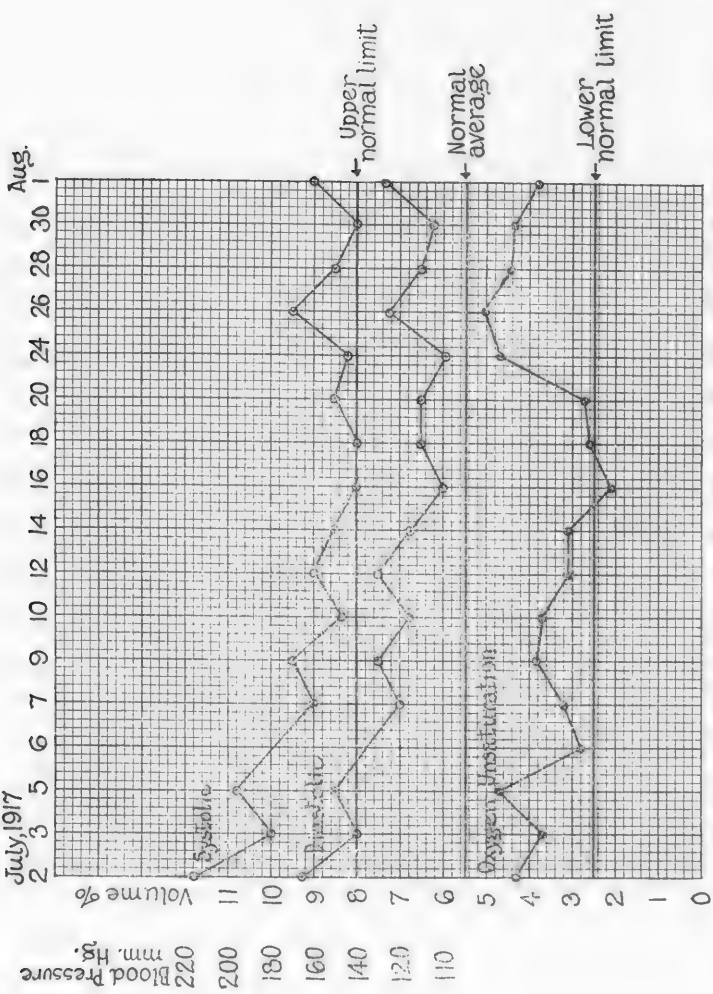
veins; heart covered by the lungs; limits uncertain at percussion; x-ray shows the heart moderately enlarged to the left; no murmurs; aortic second sound high pitched and accentuated. The abdomen is fat with prominent veins on both sides from the inguinal region to the axilla. Liver not felt; area of dullness over spleen not distinctly increased; no jaundice; no ascites; no edema; the urine con-



TEXT-FIG. 2. Oxygen unsaturation of venous arm blood. Case of arterial hypertension; slight arteriosclerosis; cirrhosis of liver; lues.

tains a faint trace of albumin; no casts; blood urea not increased. Wassermann reaction positive. Temperature normal.

Treatment.—Confined to bed; restricted fluid and calories; no medication apart from antisyphilitic treatment, which was started after the determinations of the oxygen were finished.



TEXT-FIG. 3. Oxygen unsaturation of venous arm blood. Case of arteriosclerosis; hypertension; lues; aneurysm; aortic insufficiency.

TABLE III.

Case 3. Arteriosclerosis; Hypertension; Lues; Incipient Aneurysm of the Aorta.

Determination No.	Bleeding.				Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a − v).	Pulse.	Respirations.
	Date.	Hour.	Arm.	Condition.	Sample 1.		Sample 2.		Average (v).					
					Hour.	Result.	Hour.	Result.						
	1917					*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	July 2	2.00 p.m.	Left.	1 hr.	2.10	17.14	2.30	17.32	17.23	116	21.48	4.25	108	20
2	" 3	9.30 a.m.	"	In bed.	10.00	17.63	10.30	17.86	17.75	116	21.48	3.73	80	14
3	" 5	11.30 "	"	" "	12.00	16.46	1.00	17.02	16.74	116	21.48	4.74	88	20
4	" 6	9.50 "	"	" "	4.40	18.64	5.20	18.64	18.64	116	21.48	2.84	88	20
5	" 7	9.20 "	"	" "	9.40	18.24	10.20	18.44	18.34	116	21.48	3.22	94	24
6	" 9	11.00 "	"	" "	11.50	18.44	12.00	18.22	18.33	120	22.20	3.87	80	20
7	" 10	4.30 p.m.	"	" "	5.30	15.56	6.00	18.54	18.55	120	22.20	3.65	80	22
8	" 12	9.20 a.m.	"	" "	11.00	18.95	11.20	20.05	19.50	122	22.54	3.04	80	22
9	" 14	9.40 "	"	½ hr.	11.00	17.45			17.45	111*	20.54	3.09	81	20
10	" 16	9.30 "	"	½ "	10.00	18.44	10.30	18.50	18.47	111	20.54	2.07	86	24
11	" 18	9.50 "	"	½ "	11.30	16.86	11.50	17.30	17.08	106	19.62	2.54	84	24
12	" 20	9.30 "	Right.	½ "	9.50	17.03	10.20	17.24	17.14	107	19.80	2.66		
13	" 24	9.30 "	Left.	½ "	11.00	14.53	11.30	14.53	14.53	104	19.25	4.72	88	26
14	" 26	9.30 "	"	½ "	10.50	14.22			14.22	104	19.25	5.03	96	20
15	" 28	9.30 "	Right.	½ "	9.50	14.84			14.84	104	19.25	4.41	72	20
16	" 30	9.30 "	Left.	½ "	9.50	13.92	10.30	14.34	14.13	100	18.50	4.37	80	24
17	Aug. 1	9.30 "	Right.	½ "	9.50	14.88	10.30	14.54	14.71	100	18.50	3.79	94	24

*The determination of the hemoglobin was repeated three times on the blood in which the oxygen was determined. Furthermore, a sample was drawn from the ear, which gave the same result. The standard was also tested.

This patient was a moderately advanced case of the cardiovascular type. Besides he probably had some cirrhosis of the liver. His circulation was fully compensated. The oxygen unsaturation shows less constancy than in Case 1. However, the variations extend practically only over the half of the area which is covered by the values for the normal individuals. The variations are smaller than in the normal person and the curve as a whole is more uniform.

Case 3 (*Table III*, *Text-Fig. 3*).—L., male, musician; age 35 years.

Diagnosis.—Arteriosclerosis; hypertension; lues; incipient aneurysm of the aorta.

Previous History, Symptoms, etc.—Headache; shortness of breath on exertion; contracted syphilis 6 years ago; treated regularly.

Physical Examination.—The site of the heart is normal to percussion and x-ray; no murmurs; both aortic and pulmonic second sounds very distinct; aortic particularly high pitched; heart action regular; electrocardiogram normal. The x-ray picture shows a slight distention of the left side of the upper part of the descending aorta. Wassermann test doubtful three times. Lungs clear; no cyanosis, distended veins, or edema; liver not felt; radial and temporal arteries tortuous and very hard; urine negative for albumin. For the blood pressure see Text-fig. 3. Temperature normal.

Treatment.—Confined to bed for the first 12 days; salt-free diet; restricted calories and fluid.

During his stay in bed his hemoglobin increased from 116 to 122. The 2nd day he was out of bed 3 hours, his hemoglobin dropped suddenly to 111 (verified several times on two different samples of blood) and then slowly down to 100 per cent. No explanation for this could be found in his condition or treatment.

We have here a patient with a pronounced premature arteriosclerosis, probably on syphilitic basis. Besides that he had a beginning aneurysm which could be detected only by x-ray. His shortness of breath on exertion is probably due entirely to this high blood pressure. The values for the oxygen unsaturation in seventeen determinations are rather constant and, like the values in Case 2, occupy the space below the normal average, one a trifle below the normal lower limit.

Case 4 (*Table IV*, *Text-Fig. 4*).—J. A., male, porter; age 49 years.

Diagnosis.—Cirrhosis of the liver; nephritis; urea retention; uremia (?); anemia.

Previous History, Symptoms, etc.—Edema of legs for 3 to 4 months; has used much alcohol; has never had any sickness before.

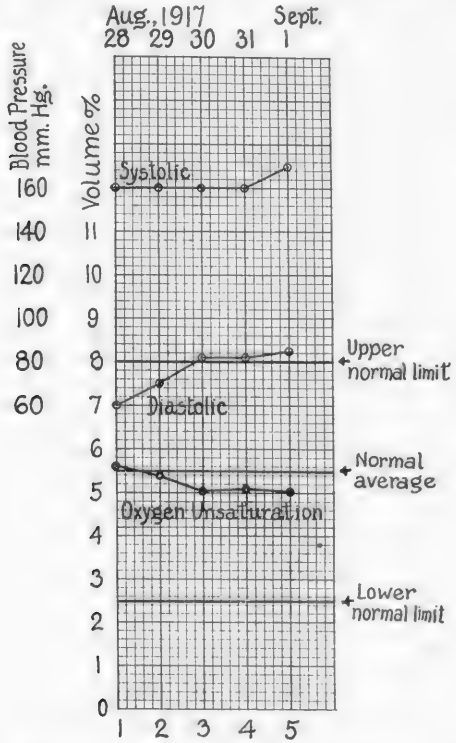
Physical Examination.—Dullness from the right border of sternum to 2 cm. below the nipple. X-ray shows that the heart is in a transverse position (on account of his ascites); the size and form not changed. All over the precordia a

TABLE IV.

Case 4. Nephritis; Uremia; Cirrhosis of Liver; Anemia.

Determination No.	Bleeding.				Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a - v).	Pulse.	Respirations.
	Date.	Hour.	Arm.	Condition.	Sample 1.		Sample 2.		Average (v).					
					Hour.	Result.	Hour.	Result.						
	1917					*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	Aug. 28	9.30 a.m.	Right.	In bed.	9.50	7.80			7.80	72	13.32	5.52	79	22
2	" 29	9.30 "	"	" "	9.50	7.90	10.10	7.90	7.90	72	13.32	5.42	78	22
3	" 30	4.00 p.m.	"	" "	4.10	8.16	4.30	8.36	8.26	(72)	(13.32)	5.06	94	26
4	" 31	10.00 a.m.	"	" "	10.20	7.85	10.40	7.85	7.85	70	12.94	5.09	78	28
5	Sept. 1	6.50 "	"	" "	8.20	8.28	8.40	8.28	8.28	72	13.32	5.04	96	24

soft systolic murmur;[2] aortic and pulmonic second sounds normal; pulse regular; electrocardiogram normal; the day of the first determination some moist râles in lungs; since then clear; no cyanosis; veins are not distended; marked ascites; no jaundice; liver and spleen not felt, but the area of dullness over the spleen is distinctly increased; uremic odor of breath; sleepy; heavy edema of lower extremi-



TEXT-FIG. 4. Oxygen unsaturation of venous arm blood. Case of cirrhosis of liver; nephritis; uremia; anemia.

ties extending up over the lower part of abdomen; diuresis small. Urine contains albumin; granular and hyaline casts; urea in blood somewhat increased (50 mg. of urea per 100 cc. of blood). Condition stationary during the time of bleeding.

Treatment.—Confined to bed; 800 cc. of milk per day. Diuresis very small (200 to 400 cc.). For the blood pressure see Text-fig. 4.

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[2] He was admitted as a case of incompensated heart failure.

The diagnosis in this case was confirmed by an autopsy which showed a typical liver cirrhosis of Laennec's type, a big hard spleen, parenchymatous, subchronic nephritis; heart and lungs were normal; no sclerosis of aorta. The results on this patient show the importance of using the oxygen unsaturation, not the direct value for the oxygen in the venous blood, on account of the anemic condition. A consideration of the whole clinical picture made it obvious that his ascites, edema, and low diuresis were not due to incompensated circulation. It is, therefore, interesting, to find normal values for the oxygen unsaturation. The values are not only within the normal limits, but they are very constant and follow absolutely the line for the normal average. The day when the first determination was made quite a number of moist râles were heard at the base of the left lung. At the time of the following determinations the lungs were clear. However, no differences were observed in the figures for the oxygen unsaturation.[3] He was very quiet all the time, in fact his mind was not quite clear, particularly at the time of the last determinations. The temperature was normal. He died 5 days after the last determination.

Case 5 (*Table V*, *Text-Fig. 5*).—G., male, teacher; age 38 years.

Diagnosis.—Mitral insufficiency; cardiac palpitations; auricular fibrillation.

Previous History, Symptoms, etc.—Palpitation; fatigue on exertion, for 4 to 6 years; no rheumatic fever; no syphilis.

Physical Examination.—On admission moist râles at the base of the left lung; otherwise lungs are clear; no cyanosis or swelling of superficial vein; dyspnea only on rather considerable exertion; dullness distinctly increased to the left and in direction of the left axilla; at apex distinct systolic murmur replacing almost entirely the first sound; no diastolic murmur; pulse rate moderately rapid (100); electrocardiogram and pulse show auricular fibrillation; liver not felt, not tender; no edema. Temperature normal. From April 19 to 30 he had 0.5 gm. of digipuratum a day. He did not show any clinical signs of incompensations. The râles in his lungs disappeared and the lungs were clear at the time of the determinations; temperature normal.

The results show two very important traits. In the first place, the values for the oxygen unsaturation vary to an extent not seen before, from 4.5 to 16.2 volume per cent. In the second place, he is the first patient with a clinically compensated heart lesion in which there have been found values for the oxygen unsaturation outside the normal limits, 8 and 2.5 volume per cent. The results on this patient are in striking contrast to those found in the other four patients.

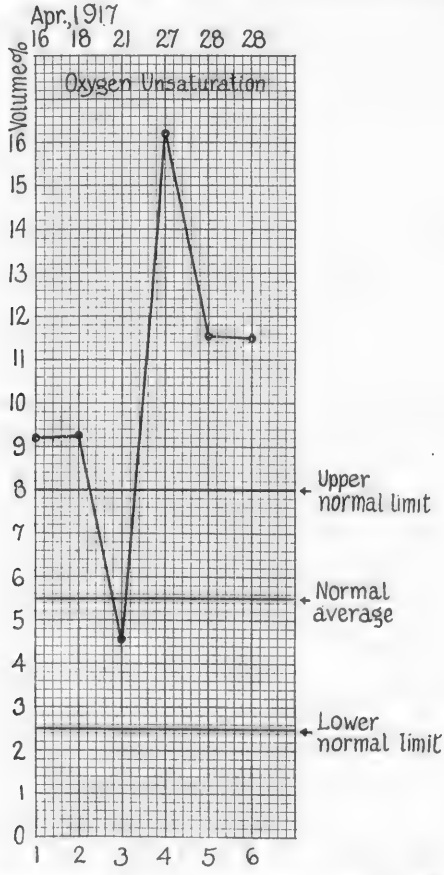
[3] See the discussion in Paper II (2) about the saturation of the arterial blood.

TABLE V.

Case 5. Mitral Insufficiency; Cardiac Palpitations; Auricular Fibrillation.

Determination No.	Bleeding.				Oxygen content of venous blood.					Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a – v).	Pulse.		Respirations.
	Date.	Hour.	Arm	Condition.	Sample 1.		Sample 2.		Average (v).				Apex.	Radial.	
					Hour.	Result.	Hour.	Result.							
	1917					*vol. per cent*		*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*			
1	Apr. 16	3.00 p.m.	Right.	In bed.	3.40	10.00	3.55	10.08	10.04	104	19.24	9.20		84	20
2	" 18	11.30 a.m.	"	" "	12.10	9.91	12.30	10.25	10.08	104	19.30	9.22		64	20
3	" 21	11.40 "	"	2 hrs.	2.40	15.71	2.55	15.75	15.73	118	21.28	4.55	87	72	19
4	" 27	3.10 p.m.	"	1 hr.	3.30	5.81	3.50	6.25	6.03	120	22.20	16.17	74	64	20
5	" 28	11.00 a.m.	Left.	3 hrs.	12.00	9.01	12.30	9.27	9.14	114	20.70	11.56	86	84	22
6	" 28	11.15 "	"	3 "	12.15	9.27	1.30	9.11	9.18	(114)	(20.70)	11.52	86	81	21

The most conspicuous clinical difference between him and the other four patients is the heart rhythm. It is difficult to find other cause for the change in the oxygen unsaturation than variations in the output from the heart. The lungs were clear, there was no evidence of any



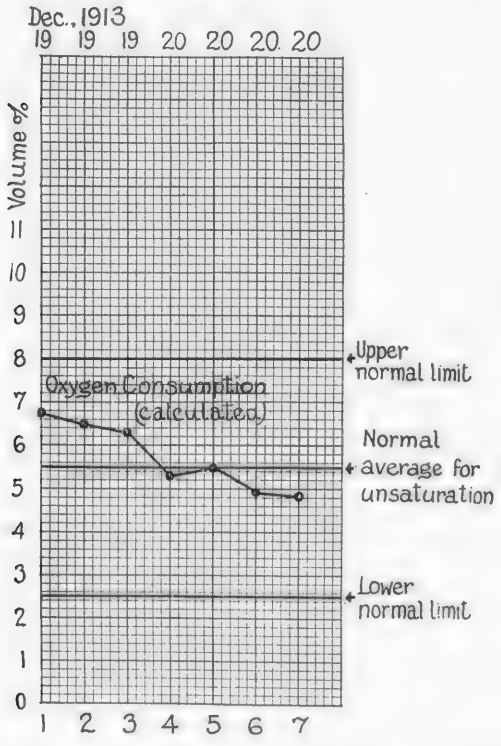
TEXT-FIG. 5. Oxygen unsaturation of venous arm blood. Case of mitral insufficiency and auricular fibrillation.

changes in the metabolism, and it seems unlikely that undetected vasomotor changes could account for the fluctuations shown in the chart.

DISCUSSION.

A previous investigation of the minute volume in heart patients by the writer (3) gave results which agree closely with those found here.

It was found that patients with valvular disease combined with arrhythmia perpetua or with groups of extrasystoles may show (1) abnormally great variations in the output and (2) diminished minute volume without clinical signs of incompensation. It is possible to calculate the degree of oxygen consumption[4] in the patients referred



TEXT-FIG. 6. Case of compensated mitral and aortic insufficiency and mitral stenosis. Pulse regular.[5] Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

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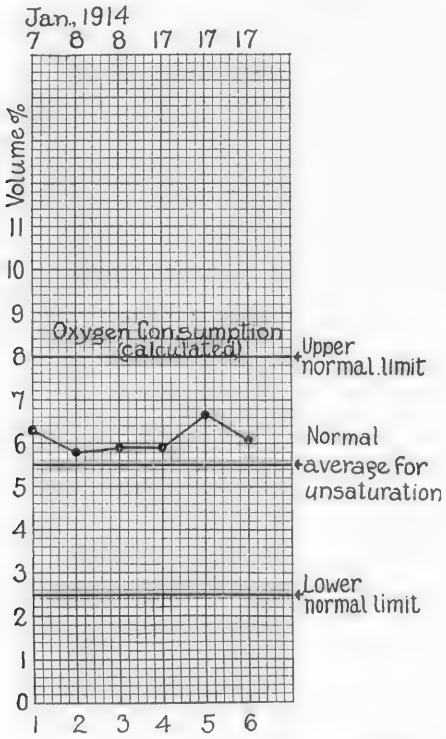
[4] The difference between oxygen unsaturation and oxygen consumption is defined in Papers I and II (1, 2). In Paper II is given a short description of Krogh and Lindhard's method.

[5] Case 1, Lundsgaard (3), p. 518.

to. Text-figs. 6 to 9 show the calculated values for the oxygen consumption in four patients where the minute volume was determined by Krogh and Lindhard's nitrous oxide method. Text-figs. 6, 7, and 8 represent patients suffering from compensated heart lesions. The

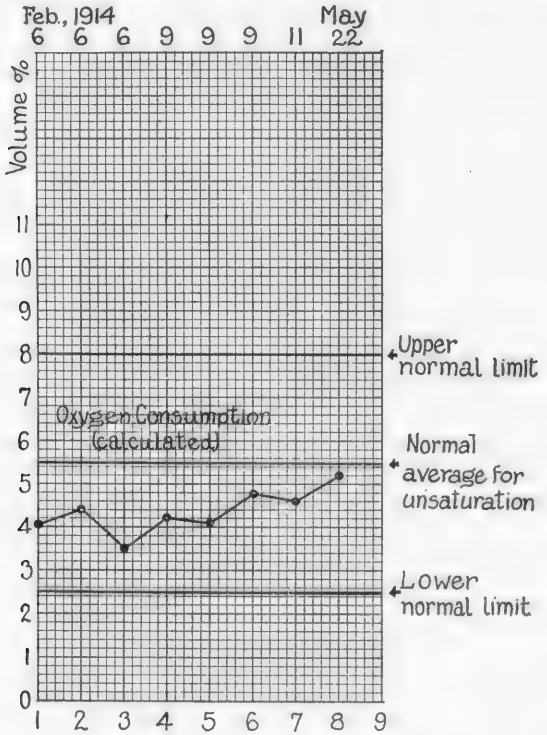


TEXT-FIG. 7. Case of compensated mitral stenosis and insufficiency. Pulse regular.[6] Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

pulse rate was regular in these cases. It will be seen that the figures agree closely with the directly determined oxygen unsaturation in the first four patients in this paper.

[6] Case 10, Lundsgaard (3), p. 544.

Text-fig. 9, on the other hand, represents a patient with clinically compensated mitral stenosis and auricular fibrillation. The calculated values for the oxygen consumption (1) are varying, (2) are above the normal line, and (3) agree closely with the directly determined

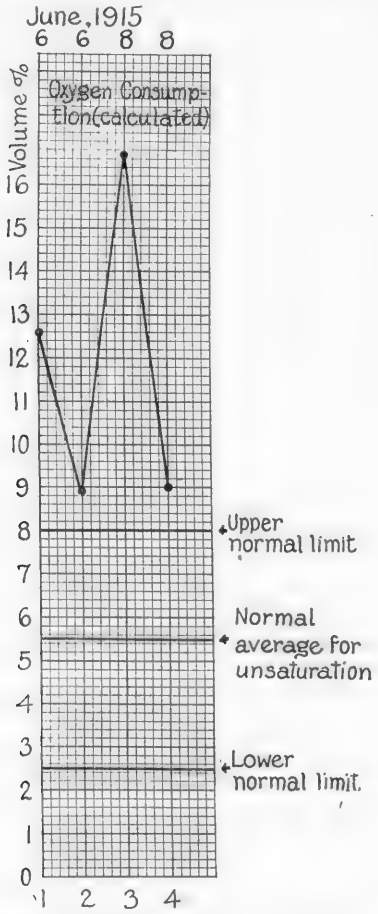


TEXT-FIG. 8. Case of compensated heart block.[7] Pulse regular. Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

values in Case 5 of this report. This seems to be sufficient proof that the variations in the oxygen saturation of the venous arm blood may in some instances express the variations in the output of the heart.

[7] Case 1, Lundsgaard (4), p. 488.

The values for the oxygen unsaturation in the first four patients are, as mentioned before, more uniform than the values in normal



TEXT-FIG. 9. Case of compensated mitral stenosis. Pulse irregular. Auricular fibrillation.[8] Oxygen consumption calculated from blood flow determinations with Krogh and Lindhard's nitrous oxide method. Lines indicate the normal average and the normal extremes for oxygen unsaturation.

[8] Case 7, Lundsgaard (3), p. 535.

people. The same thing could be seen in the determinations of the minute volume of the heart in normal people and in patients with compensated circulatory disturbances. The figures for the oxygen unsaturation calculated from the blood flow were more regular in patients with compensated valvular disease and regular heart action than in the two normal people. This may be interpreted as indicating that a regularly beating heart working against a certain burden will have a smaller margin of action than the normal heart. The burden probably acts on the heart like a heavy wheel on a machine, making its action steady.[9]

SUMMARY.

1. Forty-six determinations of the oxygen unsaturation, *i.e.*, the difference between the venous oxygen and the total oxygen capacity of the hemoglobin, have been done in five patients with compensated circulatory disturbances.

2. Values within normal limits and near or below the normal average were found in four patients with regular pulse.

3. In one patient with mitral insufficiency and arrhythmia perpetua extremely varying values were encountered. Six determinations were made; in one instance the value was within normal limits, in five above, and in one of those even higher than in most cases of incompensated heart lesions.

4. A comparison is drawn between the directly determined oxygen unsaturation in these patients and the oxygen consumption calculated from previous experiments by the writer, where the blood flow was determined directly by the nitrous oxide method. A close parallelism is found.

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4. Lundsgaard, *Deutsch. Arch. klin. Med.*, 1916, cxx, 481.

[9] It is worth mentioning that the normal person on whom twenty determinations of oxygen were done (Paper I and Text-fig. 1, Paper II) had a very unsteady circulation (unsteady pulse, respiratory arrhythmia, dermographism). Great variations in the oxygen unsaturation were encountered.

STUDIES OF OXYGEN IN THE VENOUS BLOOD.

IV. Determinations on Five Patients with Incompensated Circulatory Disturbances.

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INTRODUCTION.

The technique of drawing and preserving the blood, the results on normal individuals, and a preliminary study of patients with circulatory disturbances have been reported in Papers I and II (1, 2) of this series.

In accordance with the viewpoints laid down in these two papers an investigation of the variations in the oxygen unsaturation[1] of the venous blood over a longer period in the same person was taken up. Ten patients suffering from different forms of circulatory disturbances have been studied. In Paper III (3) a report was made of 46 determinations on five patients in a compensated stage. The present paper is a report of 103 determinations on five patients with different heart lesions, all of whom, during a certain period, were distinctly clinically incompensated. Repeated determinations have been made during the course of the disease, and the clinical symptoms which have bearing upon the compensation or incompensation have been noted.

Short descriptions of the history, the physical examination at admission, and the treatment are given. The development of the disease can be followed from the notes on the charts. In the diagrams are given the curves of the oxygen unsaturation of the venous blood.

[1] The term oxygen unsaturation means the difference between the total oxygen-combining power of the hemoglobin and the oxygen in the venous blood. For further details see the discussion in the preceding papers, especially Paper II, Text-fig. 2.

The extreme normal limits and average normal are indicated on the diagrams for comparison. Marks showing the treatment with digitalis and the condition of the circulation (compensation or incompensation) are added. The clinical symptoms, which have been especially followed, are dyspnea, cyanosis, swelling of the superficial veins, enlargement of the liver, edema, diuresis, and body weight. The figures for the respiration indicate the rate just after the blood has been drawn. For that reason the rate of respiration is sometimes somewhat higher than would be the case if the patient had not been bled. This holds particularly true in the compensated stage.

The pulse has been counted during the bleeding. In patients with auricular fibrillation the apex rate and radial rate have been counted simultaneously in order to obtain the pulse deficit. In the few instances where the temperature of the patients has been increased, it is noted in the description of the patient.

CASES.

Case 1 (*Table I, Text-Fig. 1*).—P., male, photographer; age 46 years. Admitted March 26, 1917. Discharged April 30.

Diagnosis.—Myocarditis; arteriosclerosis; chronic nephritis; hypertension.

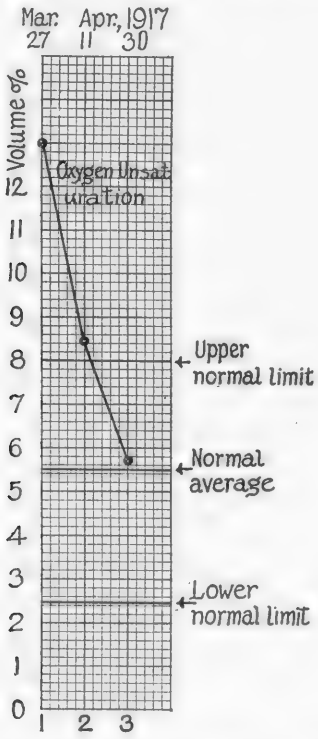
Previous History, Symptoms, etc.—Shortness of breath for a year, particularly on exertion; swelling of legs and abdomen for 2 months. No syphilis or rheumatic fever. He has used alcohol every day for several years.

Physical Examination.—Heart dullness and shadow on x-ray picture increased to the left and downwards. Limit of dullness is 3.5 cm. to the right and 14 cm. to the left of the middle line at the fourth interspace, 15 cm. to the left at the sixth interspace. An indistinct systolic murmur is heard all over the precordium. Aortic second sound is accentuated. Blood pressure: systolic 175, diastolic 125. There are moist râles and slight dullness at the lower parts of both lungs. He is cyanotic and the veins of the neck are distended. There is distinct ascites; the liver is felt, not tender. Marked edema of both legs extending up over sacrum. In the urine was found slight albumin, and occasionally granular casts. Blood urea considerably increased (73 mg. per 100 cc.). Index of urea excretion (McLean (4)) 15.0, in the course of the treatment increasing to 74.0. Wassermann test negative.

Treatment.—He was confined to bed and treated first with milk diet, and later with salt-free diet.

Three determinations of the oxygen unsaturation were done. The first was done the day after admission. He was distinctly incompensated and the value for the unsaturation was far above the upper normal

limit. The second determination was done at a time when it was doubtful whether he was compensated or not (Table I and Text-fig. 1). The oxygen unsaturation was at that time just above the upper normal limit. At the time of the third determination he was absolutely compensated



TEXT-FIG. 1. Oxygen unsaturation of venous arm blood. Case of myocarditis; arteriosclerosis; chronic nephritis; hypertension.

at rest.[2] His oxygen unsaturation was at that time normal. The interpretation of this case is made difficult by the fact that there were quite a number of râles in the lungs at the time of the first determination, which may or may not have affected the saturation of the

[2] In the evening a slight edema around the ankles was found.

TABLE I.

Case 1. Myocarditis; Arteriosclerosis; Chronic Nephritis; Hypertension.

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a–v).	Clinical notes.‡									
	Date.	Condition.*	Sample 1.	Sample 2.†	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
1	Mar. 27	In bed.	7.29	7.74	7.52	111	20.52	13	88	26§	++	++	++	+	+++	950	310	59.0
2	Apr. 11	½ hr.	14.69	14.67	14.68	125	23.15	8.47	89	17‖	–	–	–	–	+	1,500	575	47.6
3	" 30	½ "	12.48	13.00	12.74	100	18.50	5.76	98	22¶	–	–	–	–	–	1,500	625	51.8

TABLE II.

Case 2. Mitral Stenosis and Insufficiency; Mitral Stenosis (?); Recurrent Endocarditis (?).

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a–v).	Clinical notes.									
	Date.	Condition.	Sample 1.	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
1	Aug. 30	In bed.	4.50	5.00	4.75	71	13.13	8.38	108	34**	++	++	+++	+++	+	1,000	484	38.4
2	Sept. 4	" "	4.60	4.80	4.70	70	12.94	8.24	104	32	++	++	+++	+++	+	1,000	420	38.7
3	" 7	" "	2.30	2.20	2.25	67	12.38	10.13	100	28	++	++	+++	+++	?	1,000	740	39.7
4	" 10	" "	4.94	5.12	5.03	71	13.13	8.10	94	32	++	++	+++	+++	?	1,000	676	40.2
5	" 12	" "	3.62	3.80	3.71	71	13.13	9.42	90	24	++	++	+++	+++	+	1,000	565	39.8
6	" 14	" "	4.81	4.81	4.81	71	13.13	8.32	104	32	++	++	+++	+++	+	1,000	670	40.4

* Condition indicates the length of time the patient has been resting in bed before the drawing of the blood.

† The interval between the two samples given in all the tables was generally about 15 to 20 minutes.

‡ The severity of these symptoms is indicated by means of crosses. Three stages are distinguished. Three crosses indicate a dyspnea (orthopnea) during which the patient was sitting almost straight up in bed in order to breathe. One cross means a just appreciable dyspnea, and two crosses mean an intermediate stage.

One cross for cyanosis means that only the lips were bluish, two crosses indicate that the ears and skin of the face were cyanotic, and three crosses mean a rather dark blue color of lips, face, and skin of the hands.

A just appreciable swelling of the veins in the neck and the dorsum of the hands is indicated by one cross. Distinct and prominent swelling of the veins of the neck, arms, and hands is indicated by three crosses. Two crosses represent an arbitrary medium stage.

As far as the liver is concerned, one cross means that it can be distinctly felt below the curvature; two crosses indicate that it extends 3 to 6 cm. downwards; and three crosses indicate that it extends still further, and is usually rather tender.

One cross for the edema indicates a little swelling on the dorsum of the foot around the ankles, or over the inside of the tibia. Two crosses indicate a distinct swelling of feet and legs, and three crosses indicate that the edema has extended up over the femurs and hips.

It will be understood that it has been difficult to obtain a satisfactory classification of these symptoms. A question mark indicates that in some instances it has been impossible to decide whether a symptom has been absent or present.

§ Some moist râles and slight dullness at the base of the lungs.

‖ The lungs were clear except for a few crackling râles at the left axilla.

¶ Lungs clear.

** Lungs clear at each determination.

arterial blood.[3] In several instances (Papers II and III) the oxygen unsaturation has been normal in patients, where just as many râles were heard as in this case. At the time of the second determination a few crackling râles in the left axilla were noticed. These disappeared the next day.

The edema in this case was supposed to be chiefly of nephritic origin. Besides this it was evident that he had an incompensated myocarditis. The results of the oxygen determinations in this case at the different stages of the disease agree therefore with what was previously found in patients with compensated and incompensated heart lesions (Papers II and III).

Case 2 (*Table II, Text-Fig. 2*).—M., male, student; age 14 years. Admitted August 28, 1917. Discharged October 21.

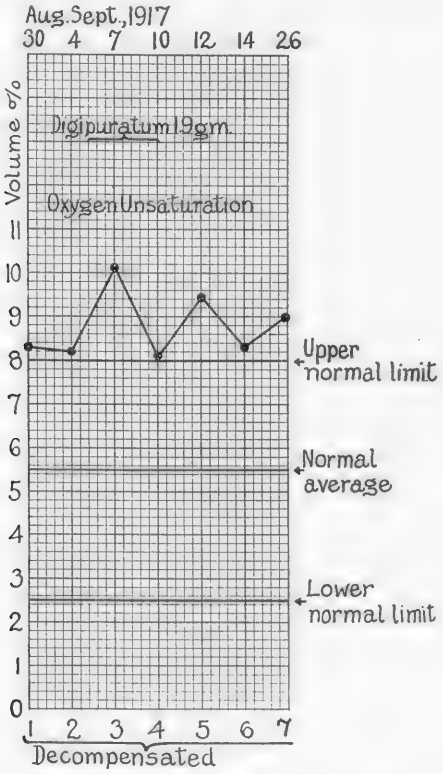
Diagnosis.—Mitral and aortic insufficiency; mitral stenosis (?); recurrent endocarditis (?).

Previous History, Symptoms, etc.—Palpitation and dyspnea, particularly on exertion, for 3 years. He has had four attacks of rheumatic fever, the first 6 years ago. The second, 3 years ago, involved his heart. He had, furthermore, an attack of chorea 2 years ago. During the last 2 months, since the last hospital treatment for rheumatic fever and endocarditis, he has suffered from increasing palpitation and dyspnea. He has been confined to bed during the last 4 months.

Physical Examination.—A few râles on deep inspiration at the base of both lungs. The râles disappeared quickly and the lungs were clear at the time of the determinations of the venous oxygen. There is distinct bulging of the precordium. No thrills. The heart is considerably enlarged to the right and left, 4 cm. to the right and 12.5 cm. to the left of the middle line of sternum. The thorax is small. The x-ray picture shows a considerable enlargement in both directions. The normal heart sounds are replaced by blowing systolic and diastolic murmurs over the precordium. In the pulmonic area the pulmonic second sound can be heard, besides systolic and diastolic murmurs. The heart action is regular and rapid. The pulse is regular and small, collapsing in type. Blood pressure: systolic 116, diastolic 65. The electrocardiogram shows regular heart rhythm and signs of hypertrophy of the left ventricle. He is moderately cyanotic, the superficial veins are distended, and he is dyspneic. Liver extends from the fifth rib to 10 cm. below the costal margin in the nipple line, and is tender. Spleen is not felt. No ascites. There is slight edema of the lower legs and more distinct edema posteriorly over the hips. The edema over the hips disappeared quickly. Diuresis small, 420 to 740 cc. In the urine is found a trace of albumin; no casts.

[3] See the discussion in Papers II and III.

Treatment.—He was confined to bed; had salt-free diet and restricted calories. From September 5 to September 10 he had digipuratum about 0.5 gm. a day, without any appreciable effect on the circulation. Besides, he had iron, arsenic,



TEXT-FIG. 2. Oxygen unsaturation of venous arm blood. Case of mitral and aortic insufficiency; mitral stenosis (?); recurrent endocarditis.

and aspirin. His clinical condition as a whole was unchanged during the determination of the blood.[4] He ran some temperature at the time of determinations of the oxygen (rectal temperature between 100 and 101°F.).

[4] September 27. A determination of the blood oxygen (not given in the table) showed an unsaturation of 9 volume per cent. His condition was the same as before. October 21. He was discharged; his clinical condition was unchanged.

This is a case of incompensated valvular heart disease on rheumatic basis. He probably has some endocarditis too. The values for the oxygen unsaturation are all above the upper normal limit. They are rather constant, all being between 8 and 10 volume per cent. The clinical condition was as a whole constant and unchanged, although the edema showed a tendency to disappear. It is important to emphasize that he was running a little temperature (100–101° F.) all the time. It is possible that this may have some effect on the oxygen unsaturation on account of increased metabolism.[5] His lungs were perfectly clear. It is interesting to note the constancy of the values for the oxygen unsaturation compared with those found in the three following cases (fibrillators). It is more constant than the values found in the normal individual (Papers I and II). The margin of action of his heart is probably very limited.[6] Digitalis was without effect—a striking contrast to what was found in the three fibrillators.

Case 3 (*Table III, Text-Fig. 3*).—Z., housewife; age 54 years. Admitted June 21, 1917. Discharged July 25.

Diagnosis.—Chronic cardiac disease (auricular fibrillation); chronic myocarditis.

Previous History, Symptoms, etc.—Shortness of breath and swelling of legs for the last 3 months. The symptoms have continuously grown more and more severe. Neither rheumatic fever nor lues.

Physical Examination.—A few râles during the last part of the inspiration on both sides from the eighth to the tenth rib. No distinct dullness. There is marked dyspnea, slight cyanosis, and distinct swelling of the superficial veins. Heart dullness from 3 cm. to the right of the middle line of the sternum to 14 cm. to the left in the fourth intercostal space. X-ray picture shows the heart moderately enlarged to the left. The heart action is very rapid and irregular. The electrocardiogram shows auricular fibrillation. No distinct murmurs are heard. The aortic and pulmonic second sounds are distinct and equal. The patient is generally a fibrillator. Blood pressure: systolic 185, diastolic 120. It is difficult

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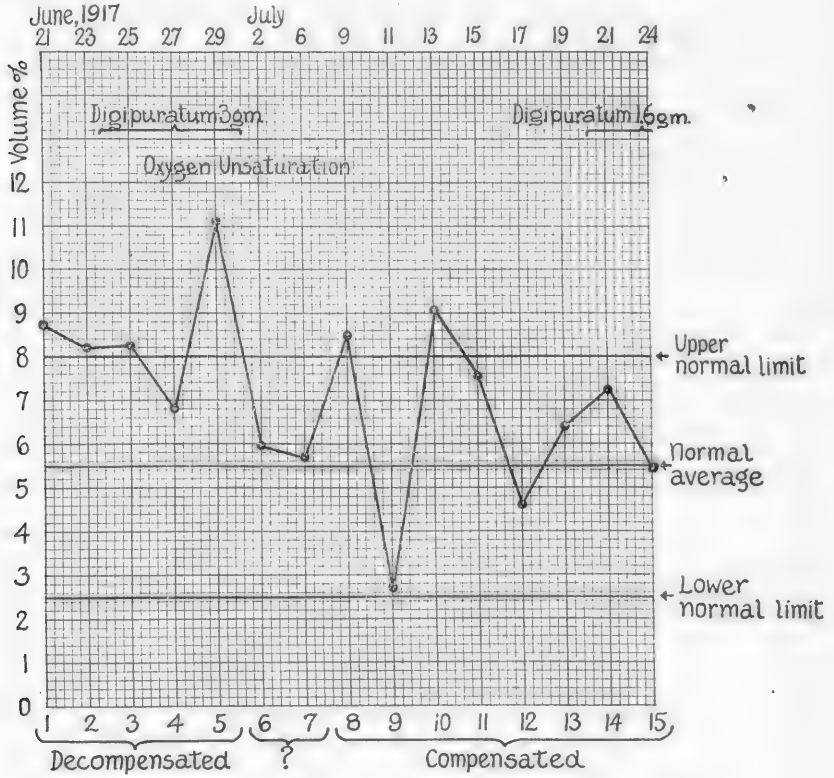
[5] See the discussion in Paper II.

[6] As mentioned in Paper III, a valvular defect (aortic and mitral insufficiency, aortic stenosis) or increased blood pressure probably influences the action of the heart in the same way that a heavy wheel influences a machine. In mitral stenosis with auricular fibrillation the opposite holds true. The reason is that the increased resistance is here offered to the auricle instead of the ventricle (see the discussion on page 559, Lundsgaard (5), where the difference between the auricular and the ventricular form of heart failure is pointed out according to the result of the blood flow determinations reported in the paper referred to).

to decide whether or not the liver is enlarged. No tenderness over the left epigastric region. Spleen is not felt. No ascites. Wassermann test negative. Urine negative for albumin. Blood urea normal. Temperature normal.

Treatment.—She was confined to bed until July 14. From that date she was



TEXT-FIG. 3. Oxygen unsaturation of venous arm blood. Case of chronic cardiac disease (auricular fibrillation); chronic myocarditis.

allowed to be out of bed, increasing the time every day. She had milk diet (800 cc.) for the 1st week. Since then salt-free diet (1,800 to 2,000 calories). Digipuratum, 0.5 gm. a day, was given from June 24 to 30 and about 0.3 gm. a day from July 20 to 25. She lost rapidly in weight from the 1st day in the hospital in spite of a rather small diuresis. Temperature normal.

TABLE III.

Case 3. Chronic Cardiac Disease (Auricular Fibrillation); Chronic Myocarditis.

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a−v).	Clinical notes.									
	Date.	Condition.	Sample 1.	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
1	June 21	In bed.	13.83	13.84	13.83	122	22.57	8.74	96* 91	44†	+++	+	++	?	+++			66.1
2	" 23	" "	12.54	12.12	12.33	111	20.54	8.21	112 87	36†	++	+	++	?	+++	1,200	635	64.6
3	" 25	" "	11.85	11.43	11.64	107.5	19.89	8.25	110 102	36‡	++	+	++	?	++	1,300	820	62.6
4	" 27	" "	13.64	13.84	13.74	111	20.54	6.80	118 87	33	++	?	++	−	++	1,355	1,955	58.6
5	" 29	" "	9.70	9.86	9.78	113	20.91	11.13	89 83	38	++	+	++	−	+	1,500	990	56.3
6	July 2	" "	15.85	15.85	15.85	118	21.82	5.97	86 83	24	?	−	?	−	?	1,500	620	54.6
7	" 6	" "	16.76	16.06	16.41	120	22.20	5.79	82 82	28	−	−	−	−	?	1,300	815	54.1
8	" 9	" "	15.08	15.08	15.08	122	22.57	7.49	110 90	28	−	−	−	−	−	1,300	770	54.0
9	" 11	" "	19.48	18.80	19.14	118	21.82	2.68	100 72	28	−	−	−	−	−	1,150	965	54.0
10	" 13	" "	11.23	11.79	11.51	111	20.54	9.03	89 86	32	−	−	−	−	−	1,400	825	53.6

11	July 15	3 hrs.	12.10		12.10	106	19.62	7.52	100/92	28	—	—	—	—	—	1,300	980	52.6
12	" 17	1 hr.	14.66		14.66	104	19.25	4.59	?									
13	" 19	1 "	12.82		12.82	(104)	(19.25)	6.43	92/68	30	—	—	—	—	—	1,300	800	53.0
14	" 21	1 "	11.26	11.02	11.14	100	18.50	7.36	94/82	30	—	—	—	—	—	1,300	400	53.2
15	" 24	2 hrs.	12.16	12.16	12.16	95	17.58	5.42	87/84	28	—	—	—	—	—	1,300	450	52.8
																1,600	400	52.5

* The upper figure is the apex pulse, the lower the radial pulse.
† A few râles at the base of the lungs.
‡ The lungs were clear from June 25 on.

This patient was distinctly incompensated at admission, but recovered quickly (Table III). The values for the oxygen unsaturation of the venous blood started above the upper normal limit. At the time of the first two determinations the patient had râles in the lungs. At the third determination the lungs were clear and remained so during the rest of the time. In spite of the difference in the condition of the lungs no variations were encountered in the oxygen unsaturation of the venous blood of the first three determinations, a fact which supports what has previously been suggested (Paper II) that râles do not necessarily influence the oxygen unsaturation of the venous blood. This cannot mean anything else than that the saturation of the arterial blood has been as complete as in individuals with normal lungs. The fourth oxygen determination shows a value within normal limits; the clinical signs of incompensation were decreasing but still to be seen. The fact that a value for the oxygen unsaturation within normal limits can be obtained in a stage of clinical incompensation does not disagree with what has been previously found in Paper II.[7] This question will be discussed in the next case.

The fifth determination shows the highest values for the oxygen unsaturation obtained in this patient. The next day it went down to the normal average. At that time it was questionable whether she was clinically incompensated or not. On July 7 it was evident that the clinical symptoms of incompensation had disappeared and from that date the oxygen unsaturation as a rule was within the normal limits. In two instances, however, it was above the upper normal limit and as a whole it was variable. The fact that we can obtain values for the oxygen unsaturation above the upper normal limit in a period of full compensation agrees with the findings in Patient 5, in Paper III, and with those in the two following patients in this paper. It is probably explained by the fact that the values for the oxygen unsaturation of the venous blood are above the upper normal limit in the incompensated period and within normal limits in the compensated period. As in other fibrillators, however, we may

[7] In this publication all the determinations with compensated cases showed values within the normal limits, whereas all the values obtained in the incompensated cases were above. See the discussion in Paper II.

find abnormally high values in the period of compensation. The relation of the curve for the oxygen unsaturation to the digitalis treatment is unmistakable, and the same is found in the following patients and discussed more in detail.

Case 4 (*Table IV, Text-Fig. 4*).—D., housewife; age 37 years. Admitted July 19, 1917. Discharged October 14.

Diagnosis.—Mitral insufficiency and stenosis; auricular fibrillation.

Previous History, Symptoms, etc.—Palpitation and shortness of breath, particularly on exertion. Swelling of legs. She had rheumatic fever 14 years ago, but had no heart trouble at that time. Never had lues. For the last 2 years she has suffered from her present symptoms, periodically, not continuously. 3 weeks ago she left a hospital in fairly good condition, but her symptoms reappeared quickly and have steadily grown worse.

Physical Examination.—No cyanosis or dyspnea. There is swelling of the superficial veins. The heart dullness is considerably increased, extending from the fifth interspace, 6 cm. to the right and 14 cm. to the left of the middle line of the sternum. The x-ray picture shows the heart shadow correspondingly increased. No thrill. A harsh systolic murmur, replacing the first sound, is heard over the precordium and transmitted upward in the direction of the left axilla. An indistinct presystolic murmur is heard at the apex. The pulmonic second sound is accentuated and reduplicated. All the sounds at the bases are indistinct. The heart action is violent and irregular. The electrocardiogram shows auricular fibrillation. Systolic blood pressure 100 (palpation). Diastolic could not be determined. Liver is felt 10 cm. below the curvature in the nipple line; tender. Spleen not felt. No ascites. Marked edema extending upward over the hips. Urine negative for albumin. Wassermann test negative.

Treatment.—She was confined to bed and treated with milk diet (800 cc.) during the first 10 days. Later she had salt-free diet (1,800 to 2,200 calories). Besides iron, she had 1.6 gm. of digipuratum from August 22 to 26 (about 0.3 gm. a day). Later she had 1.6 gm. of digipuratum from August 27 to September 10 (about 0.1 gm. a day.) Her temperature was normal during all the determinations.

At the time of the first three determinations some râles were heard at the base of both lungs. Later on the lungs were clear. Thirty determinations of the oxygen unsaturation of the venous blood were done during two periods with an interval of 2 weeks between. In spite of the incompensated condition and the râles the values for the oxygen unsaturation were within normal limits the first five times. The first four determinations gave values which almost followed the normal average, the fifth was nearer the upper limit. The results

TABLE IV.

Case 4. Mitral Stenosis and Insufficiency; Auricular Fibrillation.

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a−v).	Clinical notes.									
	Date.	Condition.	Sample 1.	Sample 2.	Average (v).				Pulse.	Respirations	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
1	July 19	¾ hr.	8.30	8.56	8.43	78	14.43	6.00	72/64	26*	−	−	++	+++	+++			68.4
2	" 20	In bed.	8.73	8.95	8.84	79	14.61	5.77	78/69	23*	−	−	++	+++	+++	1,750	333	
3	" 21	" "	9.51	9.51	9.51	82.5	15.28	5.77	89/80	20*	−	−	+	++	+++	1,025	280	
4	" 24	" "	8.33	8.73	8.53	78	14.43	5.90	72/70	20†	−	−	+	++	++	1,200	240	67.6
5	" 26	" "	8.25		8.25	82.5	15.28	7.03	66/60	22	−	−	+	++	+	1,200	820	67.8
6	" 28	" "	6.72	6.30	6.51	82.5	15.28	8.77	56/52	20	−	−	+	++	+	1,200	3,150	63.7
7	" 29	" "	11.16	10.72	10.94	84	15.53	4.59	44/44	20	−	−	?	++	+	1,200	2,825	51.2
8	" 30	" "	10.53	10.53	10.53	82.5	15.28	4.75	58/50	20	−	−	?	+	?	1,200	1,275	58.8
9	" 31	" "	11.17	11.17	11.17	82.5	15.28	4.11	48/48	20	−	−	−	?	?	1,200	630	57.2
10	Aug. 1	" "	9.11	8.73	8.92	82.5	15.28	6.36	54/52	22	−	−	−	−	−	1,200	555	55.0
11	" 2	" "	9.17	9.15	9.16	87	16.10	6.94	52/52	24	−	−	−	−	−	1,200	525	54.1

Interval of 14 days.

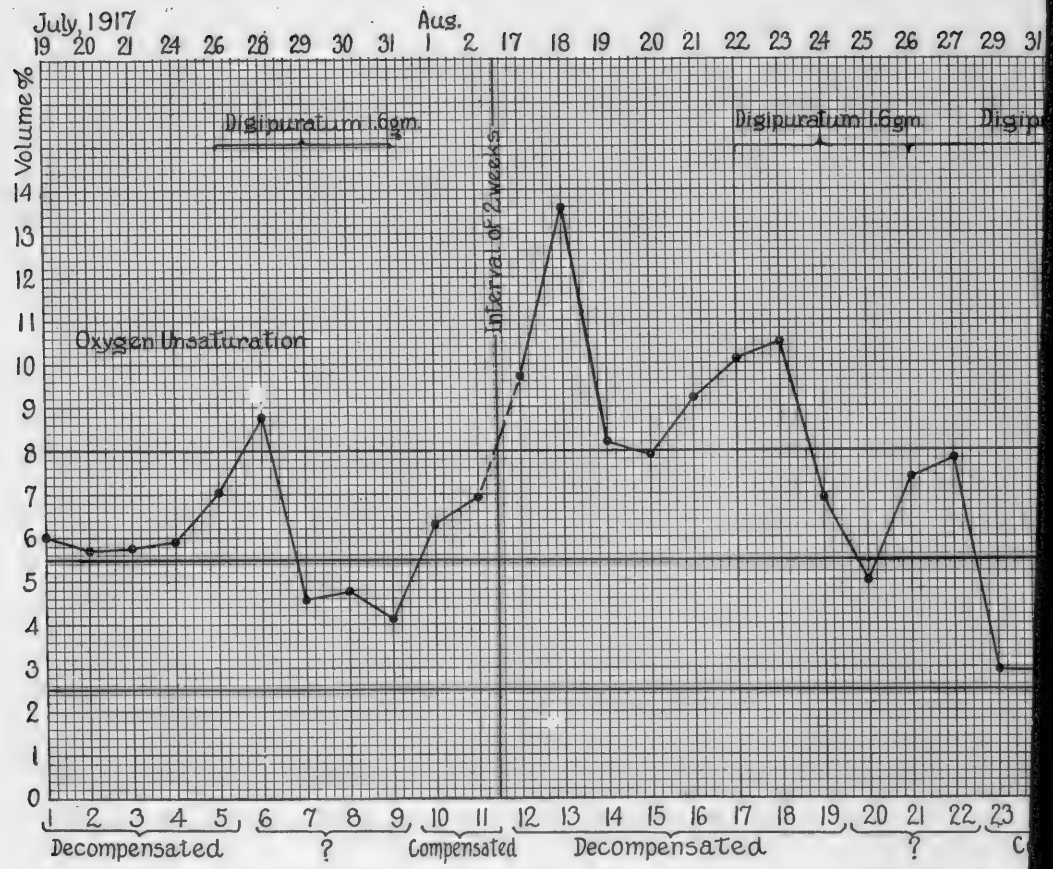
12	Aug. 17	In bed.	5.89	5.89	5.89	84	15.53	9.64	99/88	22	+	?	?	?	–	1,200	760	57.7
13	" 18	" "	1.97	2.02	2.00	84	15.53	13.53	96/92	24	++	+	+	+	?	1,200	730	57.7
14	" 19	" "	7.35	7.35	7.35	84	15.53	8.18	78/76	20	+	?	?	+	?	1,200	700	57.6
15	" 20	" "	7.40	7.84	7.62	84	15.53	7.91	78/60	20	+	?	?	++	?	1,200	615	58.2
16	" 21	" "	6.84	7.04	6.94	87	16.10	9.16	108/92	24	+	?	+	++	+	1,200	834	58.4
17	" 22	" "	6.05	6.05	6.05	87	16.10	10.05	120/84	24	+	+	+	++	+	1,200	555	58.7
18	" 23	" "	4.88	5.22	5.05	84	15.53	10.48	120/85	30	++	+	+	++	+	1,250	645	58.6
19	" 24	" "	8.55	8.13	8.34	82.5	15.28	6.94	98/88	21	+	+	?	+	+	1,200	993	59.2
20	" 25	" "	9.35	9.55	9.45	78	14.43	4.98	82/72	24	?	?	?	?	?	1,200	940	59.0
21	" 26	" "	7.16		7.16	78	14.43	7.27	?		–	–	–	?	?	1,200	1,280	59.1
22	" 27	" "	6.34	6.54	6.44	77	14.22	7.78	68/60	22	–	–	–	–	–	1,200	1,130	59.0
23	" 29	" "	11.86	11.86	11.86	80	14.79	2.93	64/64	20	–	–	–	–	–	1,200	1,000	58.6
24	" 31	" "	12.35		12.35	82.5	15.28	2.93	65/63	22	–	–	–	–	–	1,200	1,250	58.5
25	Sept. 3	" "	12.46	12.54	12.50	84	15.53	3.03	67/61	20	–	–	–	–	–	1,200	945	58.4

* Few râles.

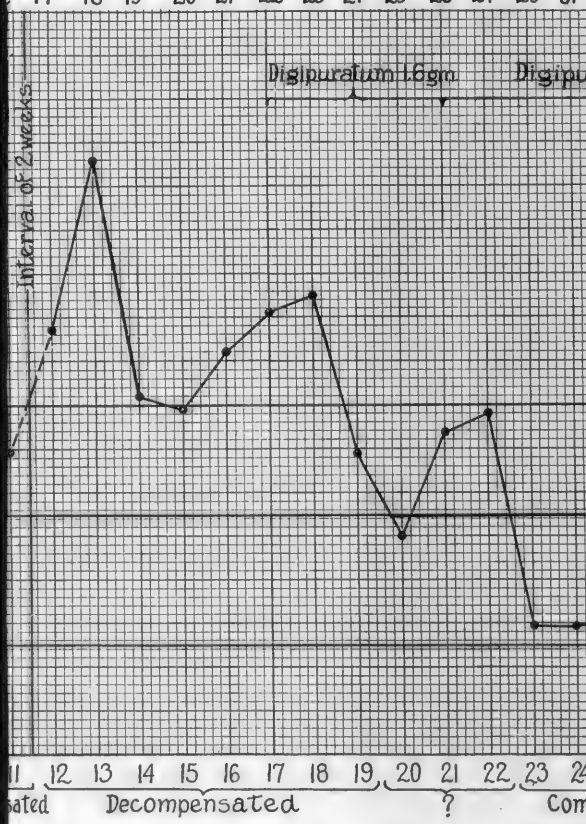
† The lungs were clear from July 24 on.

TABLE IV—*Concluded.*

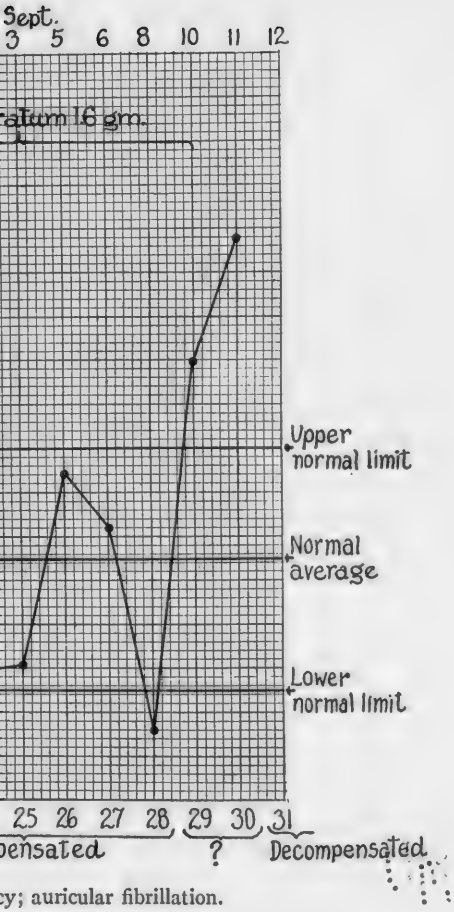
Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a—v).	Clinical notes.								
	Date.	Condition.	Sample 1.	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
26	Sept. 5	In bed.	7.82	7.82	7.82	82	15.15	7.33	72 / 66	20	–	–	–	–	–	1,200	1,875	58.7
27	" 6	" "	9.02	9.02	9.02	82	15.15	6.13	76 / 72	20	–	–	–	–	–	1,200	610	59.0
28	" 8	" "	14.20	14.20	14.20	86	15.90	1.70	80 / 80	20	–	–	–	–	–	1,200	935	59.6
29	" 10	" "	5.87	5.87	5.87	85	15.72	9.85	72 / 59	22	+	+	+	?	–	1,200	1,355	60.0
30	" 11	" "	2.70	2.70	2.70	84	15.53	12.83	76 / 62	24	+	++	+	+	–	1,200	1,240	60.3



TEXT-FIG. 4. Oxygen unsaturation of venous arm blood. Case of mitral stenosis and insuffic



of venous arm blood. Case of mitral stenosis and insufficien



cy; auricular fibrillation.

of the first five oxygen determinations show the same things which were seen once in Case 3: a normal oxygen unsaturation at a time when the patient is distinctly clinically incompensated. This is probably an expression of the fact that she was improving. It will be seen from the chart that the severity of the symptoms of incompensation were decreasing. The fourth and fifth determinations showed an increasing tendency. Digitalis treatment was started on July 26. The oxygen unsaturation was still higher the next day (No. 6), this time above the normal limit. 1 day later a sudden drop in the oxygen unsaturation (4.6 volume per cent) occurred. Whether or not this was a result of the digitalis treatment cannot be definitely decided from the present facts. Nos. 8, 9, 10, and 11 were all within the normal limits. The oxygen determinations then had to be stopped for 2 weeks. At the time of the tenth and eleventh determinations she was clinically compensated; she felt well and was allowed to be out of bed.

This clinically compensated condition lasted for about 12 days. At that time (August 14) a rather sudden change in condition occurred. The patient began to feel uneasy. She had palpitations, throbbing in the veins of the neck, and pain in the left hypochondrium. On August 17 when the oxygen determinations were taken up again she was slightly incompensated. The oxygen unsaturation (9.7 volume per cent) was above the upper normal limit. The next day it was still higher (13.5 per cent), and the clinical symptoms of incompensation were more marked. The two following determinations (Nos. 14 and 15) showed a considerable drop in the oxygen unsaturation which went down to the upper normal limit. However, an increase appeared again and the incompensation seemed to be growing. Digitalis treatment was therefore instituted (August 22). 2 days later a sudden drop occurred in the oxygen unsaturation, almost identical with what was seen at the time of the first digitalis treatment. The clinical condition now improved rapidly. On August 25 it was impossible to decide whether she was incompensated or not, and 4 days later she was again in full clinical compensation. On August 24 and 25 the same condition was encountered as at her admission: normal oxygen unsaturation despite incompensation. However, as can be seen from the table, the clinical incompensation is rapidly dis-

appearing. On August 26 the digitalis dose was decreased to 0.1 gm. a day, instead of 0.5 gm. For 10 days she felt well and was compensated in rest. On September 10 she began to feel uneasy again. Her pulse rate and pulse deficit increased, and signs of incompensation appeared. The digitalis was stopped September 11 for a week. Her clinical condition grew rapidly worse and on September 12 she was again incompensated. Corresponding to the change in the clinical symptoms, a sudden large increase appeared in the oxygen unsaturation, which by September 10 was 10 volume per cent and on September 11, 12.8, which is far above the upper normal limit. The oxygen determinations had to be stopped at that time. A week later she was given digipuratum in larger doses, 0.5 gm. a day, and the symptoms of incompensation disappeared again. The oxygen determinations in this patient show: (1) that normal values for the oxygen unsaturation of the venous blood may be found with râles in the lungs; (2) that normal oxygen unsaturation may be associated with full incompensation but improving clinical condition; (3) that an increase in the oxygen unsaturation to above the upper normal limit may precede the clinical symptoms of incompensation; (4) the results suggest the oxygen unsaturation as an indicator of the action of digitalis;[8] (5) great variations in the oxygen unsaturation are met with in this patient, as in previous fibrillators.

Case 5 (*Table V, Text-Fig. 5*).—D., male, paper maker; age 31 years. Admitted February 9, 1917. Discharged May 5.

Readmitted June 19. Discharged July 25.

Readmitted August 22. Discharged October 2.

Diagnosis.—Mitral stenosis and insufficiency; auricular fibrillation; diabetes mellitus; slight chronic interstitial nephritis.

Previous History, Symptoms, etc.—Shortness of breath for 3 years before his admission to this hospital. At 9 years of age he had rheumatic fever without heart symptoms. Denies lues. After the heart symptoms had first started 4 years ago they grew continuously worse. Besides shortness of breath and palpitation he often had swelling of the lower extremities. He has been admitted to the hospital several times (four) in an incompensated condition and every time it has been more difficult, and taken longer to obtain compensation. Be-

[8] The relation of the pulse rate and pulse deficit to the oxygen unsaturation suggests indirectly the same thing.

sides his heart disease he had a mild, not progressive diabetes and slight chronic interstitial nephritis.

Physical Examination.—The local physical signs of heart disease remained constant. At the first admission he had a considerable enlargement of the heart dullness 15.5 cm. to the right of the middle line of the sternum and 17.5 cm. to the left in the fifth intercostal space. No thrill. The x-ray picture showed corresponding enlargement of the heart shadow. At the apex a systolic and a diastolic murmur were heard. A systolic murmur was heard at the bases and the pulmonic second sound was accentuated. The pulse was irregular. The electrocardiogram showed arrhythmia perpetua. Blood pressure: systolic 180; diastolic 140. Wassermann test negative. In urine a trace of albumin, hyaline, and granular casts. Blood urea normal. Index of urea excretion fairly normal; now and then somewhat diminished. On ordinary mixed diet he would show sugar in the urine and about 0.20 per cent sugar in blood.

Treatment.—He was treated with digipuratum, antidiabetic and salt-free diet. Until he was absolutely compensated he was confined to bed. Now and then he had a slight increase of temperature (100–100.5°F.).

Forty-eight determinations of the oxygen unsaturation were done at three different periods, at the three successive admissions.

Period I.—Two determinations were done (February 17 and March 8). He had at that time been treated and the symptoms of incompensation had disappeared and he was fully compensated at rest. The values were normal (4 and 5 volume per cent).

Period II.—On June 19 he was readmitted to the hospital in a severely incompensated condition (dyspnea, cyanosis, distended veins, swelling of liver, and edema, as shown in Table V). Some râles were heard at the base of the lungs at that time. The temperature was slightly increased (100–102° F.) on the first 2 days; later normal. The determinations of the oxygen unsaturation showed considerably increased values. The first eight (the 1st week in the hospital) are all above the upper normal limit. He was at that time incompensated but improving. At the time of the first seven determinations (Nos. 3 to 9) moist râles were heard at the base of both the lungs. At the time of the eighth determination (No. 10) his lungs were clear. No difference is seen in the value for the oxygen unsaturation. The next four determinations (Nos. 11 to 14) show varying values—two above and two below the upper normal limit. A question mark on the diagram indicates that it was difficult to decide whether the patient was clinically compensated or

TABLE V.

Case 5. Mitral Stenosis and Insufficiency; Auricular Fibrillation; Chronic Interstitial Nephritis.

Determination No.	Bleeding.		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a−v).	Clinical notes.									
	Date.	Condition.	Sample 1.	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight.
									First admission.									
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
1	Feb. 17	In bed.	17.15	16.90	17.03	114	21.10	4.07	? / 67	26*	−	−	−	−	−	1,600	948	64.7
2	Mar. 8	1 hr.	14.62	14.42	14.52	106	19.62	5.10	? / 90	22*	−	−	−	−	−	1,900	1,200	71.1
									Second admission.									
3	June 20	In bed.	4.69	4.29	4.49	88.8	16.42	11.93	93 / 88	24†	++	++	++	++	+++	1,000	525	72.0
4	" 21	" "	7.87	7.41	7.64	103	19.06	11.42	? / 108	28†	++	++	++	++	+++	1,000	1,105	72.2
5	" 22	" "	10.34	10.34	10.34	100	18.50	8.16	?	−†	++	++	++	++	++	1,000	1,765	71.8
6	" 23	" "	8.15	9.07	8.61	101	18.70	10.09	? / 72	28†	++	++	++	+	++	1,000	1,243	68.8
7	" 24	" "	8.57	8.50	8.54	100	18.50	9.96	77 / 76	24†	++	+	++	+	+	1,100	1,650	66.0
8	" 25	" "	11.85	11.85	11.85	105	19.94	8.09	78 / 72	26†	++	+	+	?	+	1,100	1,760	64.0
9	" 26	" "	9.14	9.14	9.14	101	18.70	9.56	80 / 70	26†	++	+	+		+	1,100	1,485	62.4

10	June 27	In bed.	8.21	8.21	8.21	101	18.70	10.49	71/70	28*	++	+	+	?	?	1,100	1,385	60.4
11	" 28	" "	13.44	13.24	13.34	108	19.96	6.62	68/65	22*	+	?	?	—	?	1,100	1,840	60.8
12	" 29	" "	6.20	6.18	6.19	106	19.62	13.43	?	—*	+	?	—	—	?	1,100	902	58.9
13	" 30	" "	14.65	14.65	14.65	111	20.52	5.87	?	23*		—	—	—	—	1,100	1,090	58.9
14	July 1	" "	11.34	10.92	11.13	112	20.72	9.59	75/66	21*		—	—	—	—	1,100	859	58.4
15	" 2	" "	12.72	12.42	12.57	116	21.45	8.88	69/65	25*		—	—	—	—	1,100	785	58.0
16	" 3	" "	15.56	15.56	15.56	116	21.45	5.89	68/74	24*		—	—	—	—	1,400	960	57.6
17	" 5	" "	10.54	11.48	11.01	116	21.45	10.44	69/61	28*		—	—	—	—	1,400	1,080	57.6
18	" 7	" "	15.42	16.60	16.01	116	21.45	5.44	59/57	28*	As a rule dyspnea only on exertion.	—	—	—	—	1,400	1,880	57.3
19	" 9	" "	9.00	9.00	9.00	116	21.45	12.45	78/66	28*		—	—	—	—	1,400	1,950	57.4
20	" 10	3 hrs.	9.38		9.38	117	21.64	12.26	72/67	28*		—	—	—	—	1,400	1,820	57.2
21	" 12	O.N.‡	17.50	17.50	17.50	117	21.64	4.14	66/66	26*		—	—	—	—	1,400	1,620	57.2
22	" 13	"	15.55	15.77	15.66	111	20.52	4.86	64/64	26*		—	—	—	—	1,400	1,140	57.2
23	" 14	"	9.91	9.01	9.46	104	19.25	9.79	68/64	26*		—	—	—	—	1,400	840	57.2
24	" 16	½ hr.	14.08	14.52	14.30	106	19.62	5.32	78/77	30†		—	—	—	—	1,400	1,035	57.4

* Lungs clear.

† A few râles at the base of the lungs.

‡ O. N. (over night in bed) means that the blood was drawn in the morning; ½ hr. means that the patient had been in bed ½ hour before the blood was drawn.

TABLE V—*Concluded.*

Determination No.	Bleeding		Oxygen content of venous blood.			Hemoglobin (Palmer's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a−v).	Clinical notes.									
	Date	Condition.	Sample 1	Sample 2.	Average (v).				Pulse.	Respirations.	Dyspnea.	Cyanosis.	Swelling of superficial veins.	Swelling of liver.	Edema.	Fluid intake.	Diuresis.	Body weight
	1917		*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*								*cc.*	*cc.*	*kg.*
25	July 18	1 hr.	16.56	16.52	16.54	106	19.62	3.08	80/72	30*	As a rule dyspnea only on exertion.	—	—	—	—	1,400	1,140	57.5
26	" 20	1 "	13.32	13.32	13.32	106	19.62	6.30	?	—*		—	—	—	—	1,400	1,415	57.4
27	" 23	O.N.	9.46	9.46	9.46	105	19.44	9.98	?/86	24*		—	—	—	—	1,400	760	57.4
28	" 24	1 hr.	13.67	13.42	13.55	105	19.44	5.87	88/86	32*		—	—	—	—	1,400	530	57.7
									Third admission.									
29	Aug. 22	¾ hr.	11.08	11.08	11.08	111	20.52	9.44	88/78	38§	+++	+++	+++	++	+++			
30	" 22	In bed.	7.66	8.08	7.87	(111)	(20.52)	12.65	100/92	36§	+++	+++	+++	++	+++			
31	" 23	" "	10.02	10.02	10.02	110	20.36	10.34	100/78	32§	+++	+++	+++	++	+++	1,500	515	70.8
32	" 23	" "	8.92	8.92	8.92	(110)	(20.36)	11.44	108/98	34§	+++	+++	++	++	+++			
33	" 24	" "	10.17	10.53	10.35	110	20.36	10.01	96/88	44§	+++	+++	++	++	+++	1,500	680	71.4
34	" 25	" "	8.50	9.30	8.90	110	20.36	11.46	94/92	30§	+++	+++	++	++	+++	1,500	665	70.6
35	" 26	" "	8.96		8.96	106	19.62	10.66	89/74	28§	++	+++	++	++	+++	1,500	2,475	69.0

36	Aug. 27	In bed.	11.46		11.46	106	19.62	8.16	72/68	28§	++	+++	++	++	++	1,500	2,100	67.8
37	" 28	" "	9.38		9.38	107.5	19.88	10.50	160/80	28	++	+++	++	++	++	1,075	1,344	
38	" 29	" "	12.07	12.07	12.07	107.5	19.88	7.81	97/62	32§	++	++	++	++	++	1,400	315	
39	" 30	" "	12.30	11.80	12.05	107.5	19.88	7.78	74/68	32§	++	++	++	++	++	1,600	485	64.4
40	" 31	" "	4.57	4.57	4.57	107.5	19.88	15.31	67/63	32§	++	++	++	++	++	1,600	750	64.7
41	Sept 1	" "	12.65	12.65	12.65	107.5	19.88	7.23	68/64	28§	++	++	+	+	++	1,500	1,665	64.4
42	" 4	" "	4.92	4.68	4.80	106	19.62	14.82	76/64	32§	++	++	+	+	++	1,500	522	62.3
43	" 5	" "	11.52	11.72	11.62	106	19.62	8.0	74/66	30§	+	?	?	+	++	1,500	1,375	62.7
44	" 6	" "	7.67	7.67	7.67	106	19.62	11.95	78/70	32§	+	++	?	+	+	1,500	1,890	63.4
45	" 7	" "	11.25	11.25	11.25	103	19.06	7.81	73/70	28*		−	−	+	+	1,500	3,650	60.4
46	" 8	" "	14.00	14.00	14.00	104	19.25	5.25	78/64	24*	Only	−	−	+	?	1,500	2,125	58.3
47	" 10	" "	13.00	13.00	13.00	104	19.25	6.25	72/66	24*	on exertion.	−	−	?	−	1,500	2,840	55.7
48	" 11	" "	11.86	11.92	11.89	104	19.25	7.36	74/70	24*		−	−	−	−	1,500	2,935	54.7

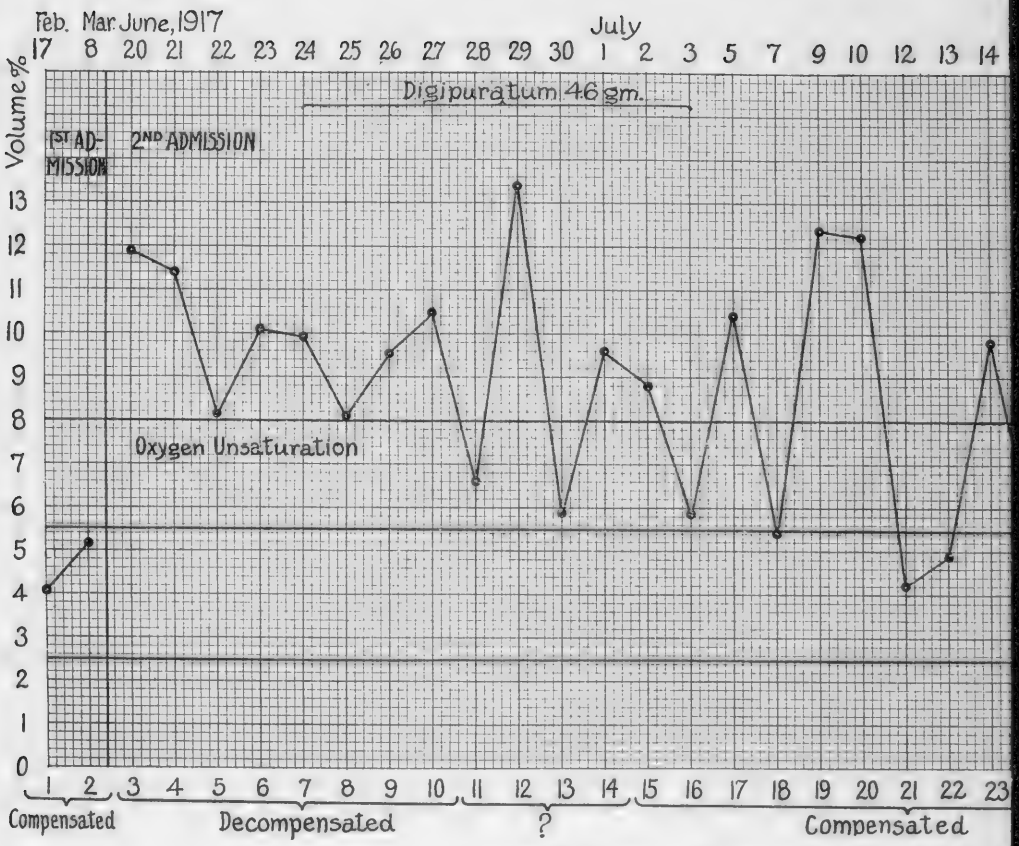
§ A few rather coarse râles at the left base; no distinct dullness.

not. On July 2 (determination 15) a period of clinical compensation at rest began. The values for the oxygen unsaturation show wide variations. Most of them are within the normal limits; a considerable number, however, are above the upper normal extreme. Upon the whole the values for the oxygen unsaturation are becoming lower and lower and there is a greater tendency to variations during the compensated than during the severely incompensated period. A line on the diagram shows the digitalis treatment (about 0.5 gm. of digipuratum a day from June 24 to July 3). It can be seen from the table that the edema decreased rapidly before the severity of the other symptoms of incompensation lessened. The same has been seen in other instances.

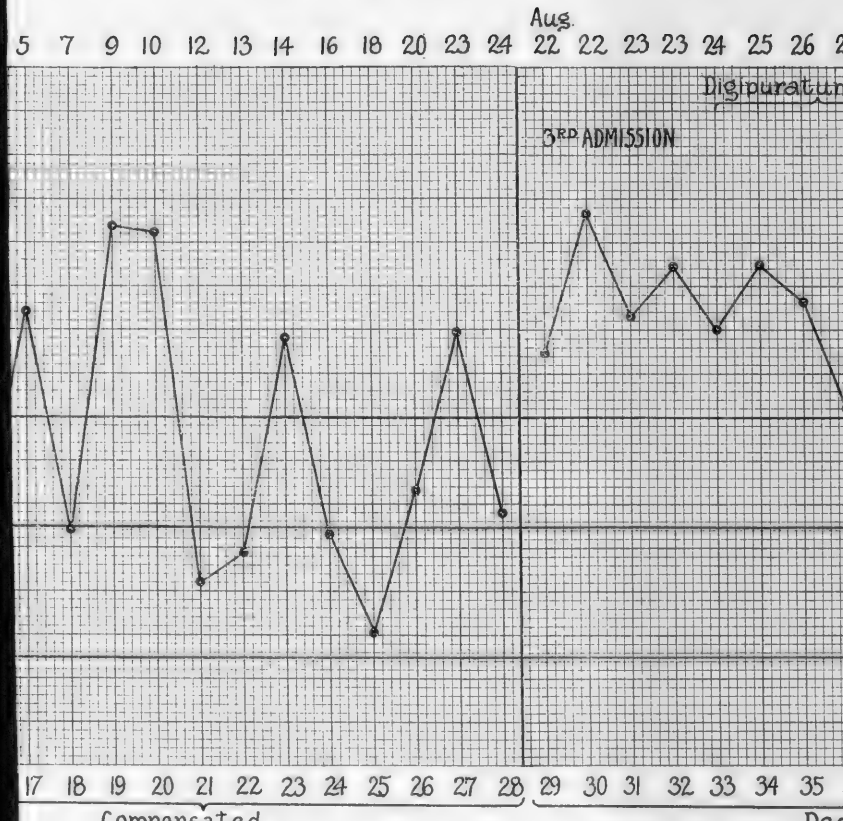
Period III.—A month later he was readmitted to the hospital. He had then for a week been in a condition of rapidly increasing incompensation which on admission was more severe than before. He was much more dyspneic and very cyanotic. The veins of the neck and arms were greatly distended and the edge of the liver was felt 10 cm. below the costal curvature in the nipple line. Heavy edema was noticed extending up his legs over the hips. The diuresis was small and he felt much distress. He was confined to bed and treated first with milk (800 cc. a day) for a week and later with salt-free diet (1,500 to 2,000 calories). From August 24 to 28 he had in all 3.4 gm. of digipuratum. It was then stopped on account of extrasystoles.[9] From September 1 to 3 he had 3 gm. of diuretin. The incompensated stage lasted this time longer than before (from August 22 to September 8). Seventeen determinations were done in this period. The first nine were all above the upper normal limit, occupying a rather limited space. The next ten were extremely variable, some of them going a little below the upper normal line; most of them, however, were far above.

His condition was only very slowly improving (Table V) until September 7 when a marked change took place. After 2 or 3 days

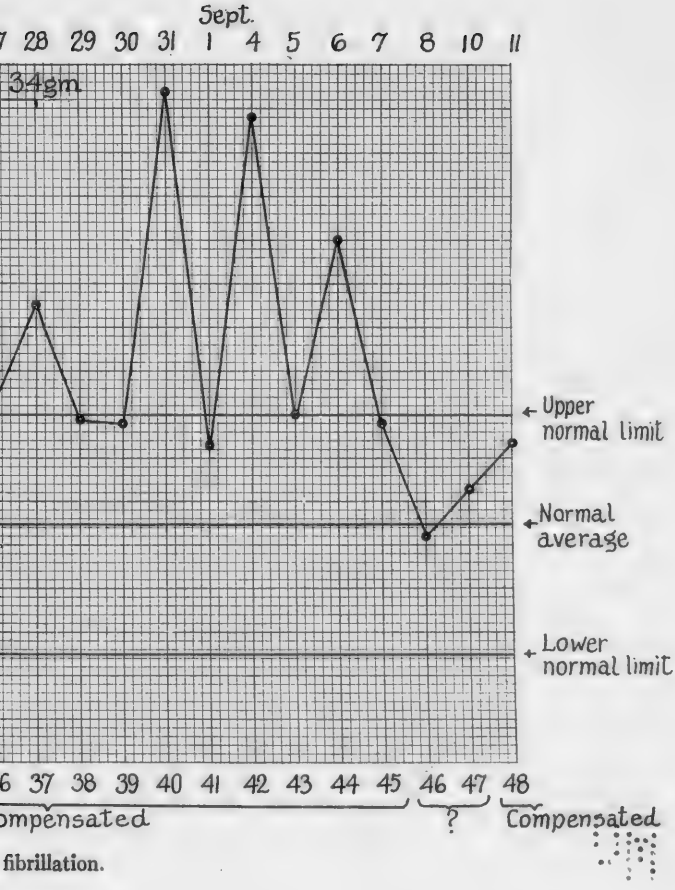
[9] The extrasystoles occurred regularly alternating with the ordinary ventricular beats. Only every other ventricular contraction was felt at the wrist. The apex rate was 160, the radial rate 80 (August 28, determination 37). The peculiar condition of the heart rhythm does not seem to have had any influence on the oxygen unsaturation of the venous blood (see Table V and Text-fig. 5).


Feb. Mar. June, 1917
July
17 8 20 21 22 23 24 25 26 27 28 29 30 1 2 3 5 7 9 10 12 13 14
Volume %
Digipuratum 4.6 gm.
1ST AD-MISSION
2ND ADMISSION
Oxygen Unsaturation
0 1 2 3 4 5 6 7 8 9 10 11 12 13
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Compensated
Decompensated
?
Compensated

TEXT-FIG. 5. Oxygen unsaturation of venous arm blo



saturation of venous arm blood. Case of mitral stenosis and insufficiency; auricular



fibrillation.

he became absolutely compensated at rest and felt extremely well. On the same date a considerable drop occurred in the oxygen unsaturation, which in the 5 following days (the last four determinations) was within normal limits. The oxygen determinations were stopped September 11. The patient was discharged October 2. He felt well and was clinically compensated. It will be seen from the table that râles were heard in the lungs from his admission (August 22) to September 6 (determination 44). From September 7 his lungs were clear. The first 4 days of his third admission he ran some temperature (100–101° F.). Thereafter his temperature was normal.

The values for the oxygen unsaturation in this patient agree as a whole with those previously found in patients with incompensated heart disease and auricular fibrillation. We can distinguish three periods in the course of the disease: (1) A period of severe incompensation. The values for the oxygen unsaturation are practically all above the upper normal limit, varying only to a moderate degree. (2) A transitory period where the symptoms of incompensation are disappearing or doubtful. In this period the values for the oxygen unsaturation are extremely variable, some above and some below the upper normal limit. (3) A period of clinical compensation at rest in which the oxygen unsaturation as a whole has a tendency to fall within the normal limits. Now and then, however, as seen in Patient 5, Paper III, the unsaturation exceeds the upper normal limit, a condition which never has been observed in compensated non-fibrillators.

DISCUSSION.

Relation of Oxygen Unsaturation to Clinical Symptoms.—As a whole, it can be said that the determinations of the oxygen unsaturation in the venous arm blood are of value in the diagnosis and treatment of patients with symptoms of circulatory disturbances. There is undoubtedly a close relation between the extent of the oxygen unsaturation and the clinical condition of the patients. As a whole, we have seen that the oxygen unsaturation in patients with compensated heart disturbances has values within the limits found in normal individuals, whereas patients with incompensated heart lesions show values above the upper normal extreme.

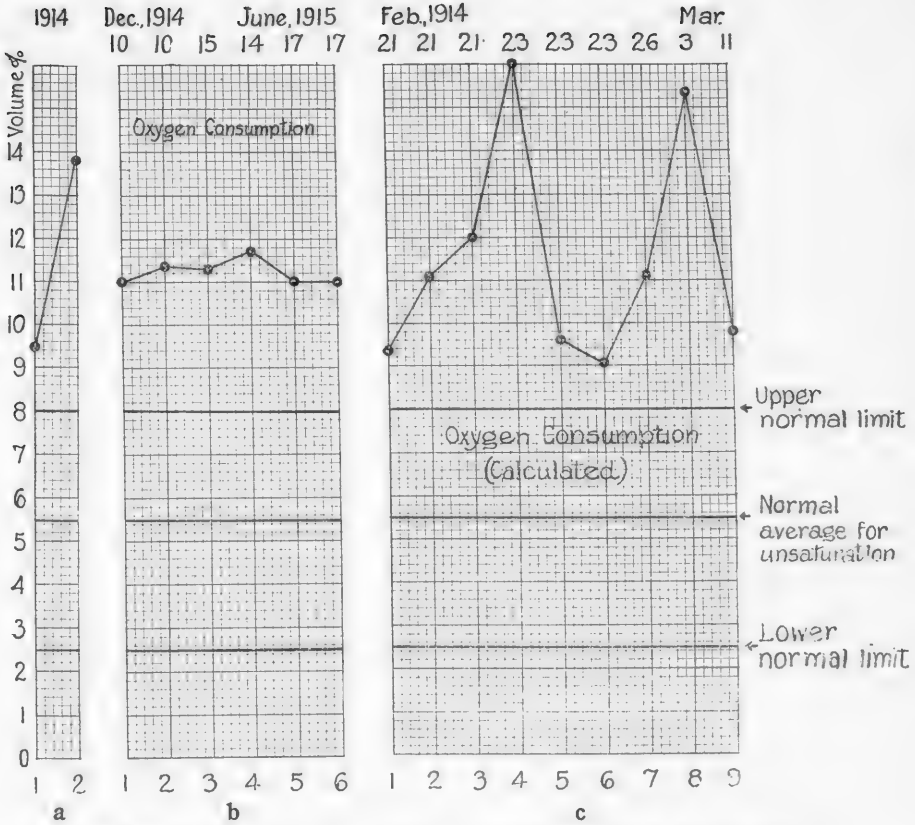
In patients in whom the incompensated condition of the circulation is decidedly improving we may find normal values for the oxygen unsaturation preceding the compensated stage, and we have seen an increase in the oxygen unsaturation to above the upper normal limit occur where incompensation was developing, but not yet clinically distinct.

In patients with auricular fibrillation the oxygen unsaturation may oscillate widely and show on some days values above the normal maximum, even when no incompensation is developing.

It is seen from the tables that the different clinical symptoms of incompensation differ in their relation to the oxygen unsaturation. Cyanosis and swelling of the superficial veins are closely related. Dyspnea appears to be usually accompanied in heart patients by increased oxygen unsaturation, but it is of course known also to occur in conditions, such as acidosis, in which the circulation is probably not retained. Edema seems to be to a great extent dependent upon special cases. It was usually possible to decrease the edema materially by the diet (salt-free) without interfering with the other symptoms and without causing any change in the oxygen unsaturation.

Oxygen Unsaturation and Blood Flow.—An attempt to calculate the rate of circulation (the minute volume of the heart) from the data obtained by the oxygen determination is not yet justified. Several facts, however, suggest that variations in the output from the heart have been by far the most important cause for the variations in the oxygen unsaturation in the patients. In Papers II and III a comparison was drawn between the directly determined oxygen unsaturation and the values for the oxygen consumption[10] calculated from blood flow determinations by means of Krogh and Lindhard's nitrous oxide method (5). A close parallelism was found, as far as the compensated patients were concerned,—even the peculiarities in the fibrillators were encountered by both methods. A similar close agreement is seen in patients with incompensated circulatory disturbance. Text-fig. 6, *a*, *b*, and *c* represents the calculated oxygen consumption in three patients with incompensation. It will be seen that all the values thus calculated on incompensated patients

[10] The difference between oxygen unsaturation and oxygen consumption is defined in Paper II.



TEXT-FIG. 6, *a*, *b*, and *c* (*a*). Case of aortic insufficiency and stenosis; mitral insufficiency; incompensated.[11] Oxygen consumption calculated from blood flow (minute volume) determinations with Krogh and Lindhard's nitrous oxide method. The normal average and the normal extremes for oxygen unsaturation are indicated.

(*b*) Case of aortic and mitral insufficiency and stenosis; auricular fibrillation.[12] Oxygen consumption calculated from blood flow (minute volume) determinations with Krogh and Lindhard's nitrous oxide method.

(*c*) Case of mitral stenosis and insufficiency; auricular fibrillation; incompensated.[13] Oxygen consumption calculated from blood flow (minute volume) determinations with Krogh and Lindhard's nitrous oxide method.

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[11] Patient 8, Lundsgaard (5), p. 537.
[12] Patient 2, Lundsgaard (5), p. 521.
[13] Patient 3, Lundsgaard (5), p. 524.

by the Krogh and Lindhard method are above the upper limit for the directly determined oxygen unsaturation in normal subjects. The patients in Text-fig. 6, *b* and *c* (Krogh and Lindhard's method) were fibrillators. The patient in Text-fig. 6, *c*, shows extremely variable values, but they are all above the upper normal limit. She was at the time of the determination in a continuously incompensated stage.

It seems, therefore, justifiable to expect that further investigations of the oxygen unsaturation in patients with different forms of circulatory and pulmonic disturbances may give us sufficient data to allow a conclusion concerning the relation of the oxygen unsaturation to the circulation.

The results on fibrillators suggest strongly that there is a qualitative difference in the dynamic pathogenesis of the heart failure in patients with normal rhythm of the heart and in patients with abnormal rhythm. This has been previously pointed out and discussed by the writer.[14]

Oxygen Unsaturation and Digitalis Action.—It seems promising to apply the oxygen determination to the study of the action of drugs, particularly digitalis, in the circulation. Cohn and Fraser have shown (6) that digitalis in a certain quantity will alter the T wave in the ventricular complex of the electrocardiogram, and have given us a valuable means of detecting the appearance of the local action of digitalis. The relation of the oxygen unsaturation to the digitalis treatment in the case reported here seems to indicate that the determination of the blood oxygen may be used as an indicator of the appearance of the effect of digitalis on the circulation as a whole.

SUMMARY.

1. A report is made of 103 determinations of the oxygen unsaturation of the venous blood of five patients with incompensated heart diseases.

2. Values for the oxygen unsaturation within normal limits were found only under two circumstances: (*a*) in a stage of full compensation, and (*b*) in a stage of incompensation where the symptoms were rapidly lessening.

[14] Lundsgaard (5), pp. 549–560.

3. Values above the upper normal extreme were met with under three circumstances: (*a*) during incompensation, (*b*) during compensation just before the clinical symptoms of incompensation had developed, and (*c*) at times in patients with auricular fibrillations in a condition of complete and stable compensation.

4. A comparison has been drawn between the directly found value for the oxygen unsaturation and the values for the oxygen consumption calculated from previous experiments by the writer on the blood flow (minute volume of the heart), in patients with similar clinical conditions. A close agreement existed.

5. It seems probable from our experience with patients under digitalis therapy that the oxygen unsaturation affords an objective criterion of the positive effect of the therapy.

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THE FACTORS CONCERNED IN THE APPEARANCE OF NUCLEATED RED BLOOD CORPUSCLES IN THE PERIPHERAL BLOOD.

I. Influence of Procedures Designed to Increase the Rate of Blood Flow through the Blood-Forming Organs—Exercise and Nerve Section.

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Plate 7.

(Received for publication, September 14, 1917.)

During the winter of 1915–16, while developing a method for isolating the circulation through the tibia of the dog (1), two of us became interested in the active vasomotor control which the vessels of the marrow were shown to possess. At this time we undertook to determine what factors of coagulation (2) arose in the bone marrow, and in order not to complicate conditions physiological salt solution, Ringer's solution, and physiological salt solution containing small amounts of sodium oxalate were used as perfusion fluids. These solutions were delivered to the bone by means of a simple constant pressure apparatus and though usually oxygenated as thoroughly as is possible with such fluids, still it must be considered that they were relatively oxygen-free. Under the conditions of the experiments it was found at once that various characteristic marrow cells appeared in the effluent. Thus, nucleated red blood corpuscles, occasional myelocytes, and even at times megakaryocytes could be identified. Our attention was directed to the question of cellular egress by the observation that vasoconstriction, produced by the addition of small amounts of adrenin to the perfusing fluid, resulted in instant diminution of the number of cells leaving the marrow, and with the characteristic vasodilatation which concludes the reaction a large increase in the number of cells was noted. These findings paralleled those made

frequently in studies of the cellular composition of blood taken from the rabbit's ear during extreme vasoconstriction and during the succeeding vasodilatation. A difference existed, however, in the fact that in this case the vasoconstriction prevented the movement of cells from their place of origin, and this at once suggested the possibility that in the marrow there existed a formative tissue with a cellular product, and that the appearance of this product, the development of leukocytosis, or the appearance of nucleated red cells in the peripheral blood, depended to some extent on control exerted by these vasomotor nerves. This supposition has been found to be questionable but has been deemed worthy of mention as it has given the basis for our attack upon the problem in hand, since if vasomotor nerves controlled the output of cells from the bone marrow they must accomplish this result by increasing and decreasing the blood flow through the tissue, this being their only conceivable function. Our experiments have indicated nothing distinctive in regard to the vasomotor arrangements in the marrow vessels, nor do the anatomical investigations of Ottolenghi (3) indicate possibilities other than those ordinarily premised in considerations of vasomotor reactions.

Increasing experience with the isolated bone marrow circulation made it evident that it would be possible, making use of appropriate perfusion apparatus (4) and using hirudinized blood, to circulate the tibial marrow thoroughly and with rates of flow which could be provided exactly. This work has been pushed forward and has been controlled by every means at our command since at best it involved radical experimental procedures. Our attention has been focussed upon the nucleated red corpuscles and our first presentations will deal almost entirely with the appearance of these cells in the peripheral blood.

It was, as has been said, our idea that increased and decreased blood flow might have something to do with cellular retention and extrusion by the bone marrow. Some ground existed for this belief. Thus, the leukocytosis of exercise is a common observation and crises of normoblasts in various anemias have been ascribed to circulatory phenomena in the marrow. In the perfusion experiments there existed a method for instant increase and decrease of blood flow through the marrow. What measures could be adopted

to achieve similar and therefore controlling results in the intact animal? Two of these are discussed in this paper; namely, exercise and section of the vasomotor supply to the four limbs. These procedures have been carried out in normal animals and in animals rendered anemic and hyperplastic as to their blood-forming tissue by repeated hemorrhages. Hemorrhage and immediate saline infusion gave the third method for dealing with the problem. It would seem that nothing could be added to the literature of this last measure; yet no accurate studies exist of the time of appearance of nucleated red cells after hemorrhage and infusion, nor has the relation of the reaction to preexisting hyperplasia been studied. It has in the past been impossible to measure the extent to which blood flow through the marrow is increased by blood dilution after hemorrhage. This has been readily accomplished by means of the isolated marrow preparation. A later paper will present the results of these experiments together with a review of former work upon this phase of the subject.

We shall give a brief review of the conditions under which nucleated red corpuscles are found in the peripheral blood, with a discussion of the marrow hyperplasias in which nucleated red cells do not enter the circulation. In most instances organs delivering a secretion must pass it through a definite channel. Once formed, this secretion can be made usable only after passage down a duct. In the bone marrow, while it seems possible to localize clearly erythrogenic groups, it has been impossible to follow the physiological act of the delivery into the circulation of young or adult cells. The character of the sinusoidal spaces into which these cells must pass is not thoroughly understood. Van der Stricht (5), Dominici (6), and Brinckerhoff and Tyzzer (7), as a result of examinations of the marrow structure in the rabbit, believe that in this mammal the endothelial walls of the sinusoidal spaces are incomplete and that the circulation consequently wanders freely among the marrow cells. It would seem physiologically impossible to keep such a tissue intact were it not protected against changes in circulatory rate with extraordinary efficiency. This last condition does not exist, for we have found it possible to increase rate and volume of flow to an extreme degree without dislocating marrow cells. It is impossible to report fully as yet upon the injected marrow, but all our preparations indicate complete vas-

cular integrity in the marrow of the dog. If this is the case, all the circulating erythrocytes in this animal must at some stage in their development pass through a membrane.

Unfortunately there is no definite histological knowledge upon the marrow circulation in the dog or in man. One is inclined to believe that the facts in regard to the marrow vessels can be obtained only by careful injections, and yet even the studies upon the rabbit, which have been accepted as picturing the typical vascular arrangement in the mammal, have not been made by this method. It is obvious, therefore, that without definite knowledge of the moves which nucleated red cells must make to reach the blood stream no final division can be made of the conditions under which they appear. The following classification of nucleated red cell appearance is, therefore, based merely upon the most significant circumstances which seem to attend extrusion.

Nucleated Red Cells from Non-Hyperplastic Marrow under the Influence of Certain Poisons.

Ricin and Saponin.—In several papers Bunting (8) has given the blood picture and marrow condition in rabbits poisoned by saponin and ricin. With the former particularly, nucleated red cells appear within $5\frac{1}{2}$ hours,—before anemia is pronounced. They do not make their appearance until anemia has been produced, however, unless there is definite marrow vessel injury evidenced by hemorrhages into the tissue substance.

While nucleated red cells are found in the blood stream in chronic arsenic poisoning, phosphorus, chloroform, mercury, and benzene poisoning, their appearance is not accompanied by the acute changes emphasized in the case of the two substances first mentioned. As far as we can find it reported, nucleated red cell appearance in chronic or acute poisoning with any of the latter group occurs in the presence of anemia and may be considered due to the anemia and not to endothelial destruction or to breakdown of the actual marrow stroma.

Appearance of Nucleated Red Cells in Relation to Certain Leukocytic Reactions.—Ewing (9) mentions that fact that "severe leukocytosis in children or even in adults may draw a few normoblasts into the cir-

culation." Roger and Josué (10) found congestion and hyperplasia in the marrow 48 hours after the injection of *Staphylococcus aureus* into rabbits. At this time hyperplasia affected the nucleated red cells and it was not until later that myelocytic increase was prominent. Rieder (11) found nucleated red cells accompanying leukocytosis some time after bacterial injections. Sherrington (12) reports the same findings during high leukocytosis induced by sterile irritation of the skin and peritoneum. Morawitz (13) has held that the tendency of nucleated red cells to appear with posthemorrhagic leukocytosis argues against the fact that oxygen lack is the stimulus causing marrow hyperplasia after hemorrhage. Since there is no synchronism between the development of this particular leukocytosis and blast appearance his argument becomes of little value. Apparently any stimulus which causes intense hyperplasia of either group of marrow cells will result in extrusion of a few members of the other variety, possibly as a result of growth pressure alone. We must realize that growth in an enclosed space demands room and that the blood spaces, the marrow sinusoids, offer the most yielding means of egress. Timofejewsky (14) injected dogs and rabbits intravenously with large amounts of septic material and within 11 minutes found many normoblasts in the circulating blood, these cells appearing in the presence of the leukopenia which immediately followed the injection. Maximum numbers of normoblasts were found 2 hours after injection. Twenty-nine experiments on dogs and rabbits are found in this series. In all cases the animals were injected suddenly with large amounts of exceedingly putrid material, and while the interpretation is not one for which definite assurance can be given, since examinations of the marrow were not made, one is inclined to believe that large injections of toxic substance may act like saponin and ricin, causing acute degenerations of the marrow.

Careful discrimination must always be made between the conditions governing acute blast extrusion in the cases just discussed and those which may readily develop in the course of severe infections. Thus, acute rheumatic fever may be attended by severe anemia, moderate leukocytosis, and the appearance of normoblasts in the circulation. But in this case there exists merely another instance of normoblast appearance in secondary anemia.

Nucleated Red Cells in Anemias.

Secondary Anemia.—That nucleated red cells enter the blood stream after single large hemorrhages or after repeated small ones has long been known. We shall at a later date present more accurate data upon the time of appearance of these cells, alterations in this time caused by repeated bleedings with resulting marrow hyperplasia, and other features of the reaction. Hemorrhage and resulting anemia will not dislocate nucleated red cells immediately, as the injections of saponin and putrid material will. There is a latent period, usually of several days, before one can be sure that the cells found have really left the marrow and have not been in the circulation before the bleeding.

Primary Pernicious Anemia and Chlorosis.—In these diseases blast appearance is irregular and there seems but one fixed feature in it; namely, that crises, first emphasized by von Noorden (15) and by Neudörfer (16), precede periods of increased red count and physical betterment. In aplastic pernicious anemias no nucleated red cells occur; in other cases they may be extremely few and yet at autopsy an intensely hyperplastic marrow may be found. Blast appearance in anemias is ordinarily the expression of a vigorous effort to regain a lost position. The loss of these young forms into the circulation is, however, an expense and simply indicates an advantageous marrow reaction without being advantageous in itself. Stockard (17) has emphasized the fact that red cell formation demands a characteristic setting whether in bone marrow, liver, or spleen, and we shall show in a later paper how thoroughly the organism is protected against the loss of young forms from the marrow even in the presence of extreme hyperplasia. Hyperplasia alone then will not cause nucleated red cell extrusion, and this introduces another set of conditions which must be considered.

Nucleated Red Cells in Conditions Not Preceded by Blood Loss, Producing Hyperplasia and Polycythemia.

We need review only the literature of polycythemia and low barometric pressure in which examinations for blasts have been made. Zuntz, Loewy, Müller, and Caspari (18) made careful observations of their own blood and of the blood of animals in their Alpine expedition. With the exception of some ob-

servations of Müller's which merit specific discussion, they never found normoblasts, though polycythemia occurred promptly and characteristically. Similarly Douglas, Haldane, Henderson, and Schneider(19) in the Pike's Peak expedition mention no nucleated red cells, though polycythemia was pronounced.

Lastly, Loevenhart and his collaborators (20) have made accurate examinations of the effects of low partial pressures of oxygen with normal and with reduced barometric pressures. They obtained polycythemia together with marked marrow hyperplasia "Blood smears from animals showing increased blood counts after exposure to atmospheres poor in oxygen when stained with Jenner stain show a number of basophilic erythrocytes. This staining reaction is characteristic of newly formed erythrocytes." There is no mention of appearance of undoubted young forms, normoblasts or megaloblasts. Loevenhart's experiments made use principally of rabbits. It is worth mentioning at this time that these animals show normoblasts in secondary anemia produced by large hemorrhages much less easily than do dogs. One dog only appears in the series in question and there is no special note upon this particular feature of the blood picture.

Among other experiments carried out at low barometric pressure we may mention first those of Gaule (21). This investigator made observations upon blood films in two balloon ascensions. The elevation reached was not great—4,200 meters at most—so that the immediate effect of oxygen lack, if this is the stimulus of high altitude, could not have been intensely acute. Yet he reports blood films loaded with normoblasts and publishes photomicrographs to illustrate this fact. We are inclined with Zuntz (18) to regard the evidence presented by these photomicrographs as due to artefacts and question the results emphatically. Schauman and Rosenqvist (22), however, in a series of experiments in which they reduced pressure suddenly in a large bell glass under which animals could be kept, an apparatus lacking, it is true, most of the refinements with which Loevenhart safeguarded his more thorough experiments, were able in a few cases to obtain evidence of sudden blast extrusion. This did not occur in all cases, but in two instances at least, within 24 hours, they found many blasts in the blood of one rabbit and one dog when previous observation had shown none. It is, however, a noteworthy fact and one which will be discussed in a later paper that nucleated red cells have no increased tendency to appear in the blood in the presence of advanced hyperplasia. Indeed, the first reaction noted in dogs subjected to repeated bleedings may be disappearance from the blood of the ordinary low normoblastic content. Something more than hyperplasia is needed to dislocate these marrow cells.

It may be held in relation to the conditions now under discussion that the stimulus is applied more slowly than we are accustomed to see it in secondary anemias due, for instance, to sudden large hemorrhages. This is true of most observations made upon human beings at high altitudes, since in these cases the experiments are not usually carried to the danger point. And it is to be remembered

that Schauman and Rosenqvist (22) by sudden reduction of pressure with no other change in the condition of the animal did find blast extrusion in two cases. Loevenhart and his associates (20) found it impossible to keep animals at oxygen percentages below 7.0. They, however, brought several animals to this minimum, kept them there a week, and include them in their statement as to the blood morphology; *i.e.*, many bluish-staining forms, no mention of blasts. The polycythemia of low oxygen tension occurs with considerable rapidity. In these experiments maximum increase was never obtained in less than 1 week, but some increase was usually evident after 2 or 3 days. Oliver (23) found immediate increase on arrival at a high altitude, and his experience is borne out by others, notably Douglas, Haldane, Henderson, and Schneider (19), Viault (24), Mercier (25), Miescher (26), and Schneider and Havens (27). It requires, however, extraordinary and dangerous application of the stimulus operative in these cases to cause blast extrusion. Another feature to be emphasized is that these cells will appear as readily and as quickly with the marrow in normal formative equilibrium as in the presence of much larger volumes of hyperplastic tissue.

Two other sets of experiments deserve notice in this connection.

Reusch (28) attempted to localize stimulation of the marrow by means of what he designated as oxygen lack. In 2 hour periods daily over a long time he tied off one leg of a dog with a rubber tube, thus cutting off all the circulation, or a large part of it, to the femur and to the tibia of the leg in question, believing that he could secure marrow hyperplasia in these bones by this method. He failed, and failed again in an attempt to get new islands of blood-forming tissue in the liver by means of hepatic artery ligation and consequent deprival of oxygen. These experiments attempt to localize stimulus and effect, a feature which is unfortunately lacking in work upon the bone marrow because of the inaccessibility of the tissue. Had this crude attempt at isolation of the tissue succeeded, the nature of the stimulus to hyperplasia might be considered to have been limited at least to some degree. While at present it appears justifiable to hold to the idea that oxygen lack achieves this result directly, we are always confronted by the fact that it may be the secondary result of oxygen lack upon other tissues.

Müller (29) has made the only investigations which we have been able to find of blood taken directly from the nutrient vein of the tibia of the dog. He isolated this vein by removal of the fibula with as little hemorrhage and interference with respiration as possible. Under these normal conditions the blood so obtained agreed in cellular composition with blood from other parts of the body. Within an hour after severe hemorrhage he found many nucleated red cells in the nutrient vein blood. Similarly these cells were obtainable after 20 minutes' clamping of the nutrient artery and after rather prolonged general asphyxia produced by breathing a nitrogen- or a carbon dioxide-rich atmosphere.

Müller attributes the appearance of these blasts to oxygen lack. He seems to show definitely that local asphyxia results in dislocation of the cells in question. There is no indication, however, as to how this acute reaction is brought

about and the observations are unfortunately given without thorough evidence of care taken in controlling the work, the manner of investigating numbers of nucleated red cells, etc. There is also no evidence as to the degree of hyperplasia of the marrow which existed when the observations were made. These are all matters of importance and may lead readily to misinterpretations of phenomena.

Müller also took part in one of the Alpine expeditions headed by Zuntz, and advantage was taken of his method for getting nutrient vein blood in the course of this group of experiments. While, as we have said, their animals showed no blasts in the peripheral blood stream, the statement is made that the nutrient vein blood was full of these cells when examined alone. The belief is advanced that nucleated forms are being continually cast into the blood stream but that they are apparently unable to survive in this free swimming condition and promptly disappear—where or how is left for conjecture. We shall show in our experiments that at times it is easy by means of exercise to change the number of normoblasts found in blood films, and we are disinclined to believe the results quoted, in the absence of evidence as to the procedures gone through before the decision of blasts in the circulation was given.

EXPERIMENTAL.

The Effect of Exercise.

The first procedure adopted to test the effect of circulatory changes in the bone marrow upon the peripheral blood of the intact animal was that of exercise. It seemed safe to assume that exercise, since it increases the flow of blood through the other organs of the body, would increase the flow through the bone marrow as well. There is no direct means of testing the effect of muscular work upon blood flow through bones since the dissection involved inevitably destroys the possibility of exercising efficiently.

The leukocytosis of exercise has already been studied. Schulz (30) in a series of observations upon men found that an increase of from 1,000 to 4,000 leukocytes per c. mm. of blood would accompany vigorous muscular effort. This leukocytosis occurred simultaneously with the increased pulse and respiration rates induced by the muscular work and, according to Schulz, disappeared in the course of 15 minutes as the pulse and respiration returned to normal.

Burrows (31) in a study of leukocytosis associated with convulsions has recorded two control experiments in which he found leukocytoses of moderate degree and short duration following vigorous exercise.

Schneider and Havens (32) in a series of 36 experiments made upon 9 subjects found an increase in the number of white corpuscles following exercise, which

varied from 1,170 to 11,670 cells per c. mm. Similarly, Hawk (33) in a series of observations on athletes has found an increase in the number of leukocytes after exercise from 1,930 to 9,500 cells per c. mm. Zuntz and Schumburg (34) in their studies on soldiers obtained an average leukocytosis, following exercise, of 43 per cent.

Larrabee, Tileston, and Emerson (35), in studies on young men before and after a Marathon race of 24 miles, record the finding of an invariable leukocytosis which varied from 14,200 to 27,700 cells per c. mm. of blood. These observers also record the fact that no abnormalities in size, shape, or coloring of red cells, and no nucleated red cells could be found. With this exception none of the above writers record observations on nucleated red blood corpuscles, the type of cell with which we are primarily concerned.

Our experiments may be considered under three main heads: the effect of exercise (1) upon normal animals, (2) upon hyperplastic animals with large numbers of normoblasts in the peripheral blood, and (3) upon hyperplastic animals showing few or no normoblasts in the peripheral blood stream.

Before discussing our results we shall review briefly our methods of making blood examinations. In selected cases specimens were obtained by withdrawing blood from a superficial vein with a hypodermic syringe, but in the majority of cases blood was obtained by subcutaneous puncture. Dogs were used as subjects throughout this series of experiments.

The examination of each specimen consisted in counting the numbers of red and of white corpuscles per c. mm. of blood, and in making blood films. The red corpuscles were diluted with Hayem's solution; the white with a 4 per cent solution of acetic acid. The same pipettes and the same Thoma-Zeiss counting chambers were used throughout and all blood counts were made by two of us. The blood films were stained with Wright's stain, and were then examined with respect to the number of nucleated red corpuscles present. In each film 1,000 white cells were counted and the number of nucleated red cells which were observed during the process of this count was recorded. Let us assume, for example, that a certain dog gave a leukocyte count of 15,000 and that the film from this animal showed 10 normoblasts per 1,000 leukocytes. The number of normoblasts per c. mm. of blood would then be estimated as being 10 times 15, or 150. This method of estimating nucleated red cells has been used with reason-

ably satisfactory results by Timofejewsky (14), by Bunting (8), and by others, and we agree with Timofejewsky in finding it more satisfactory than any method of direct counting in a counting chamber.

The Effect of Exercise upon Normal Animals.—Many investigators have noted that the peripheral blood of a large percentage of dogs normally contains nucleated red cells. Our observations amply confirm this fact. In 26 observations on the ordinary dogs used in the laboratory, before experimental procedures had been instituted, we found an average of 227 nucleated red cells per c. mm. of blood, with a range of from 0 to 2,485 cells. Of these 26 animals, 8 on first examination showed no nucleated red cells at all.

TABLE I.

Normal Dogs.

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
	1917					
1	Aug. 31	10.50	8,624,000	24,400	466	
		11.12–11.17				Vigorous exercise, running out of doors.
		11.20	8,432,000	26,500	689	
2	Aug. 29	11.50	7,440,000	14,700	0	
		11.57–12.13				Vigorous running out of doors; animal well blown.
		12.15	8,592,000	18,200	0	

In carrying out an exercise experiment the animal to be studied was kept as quiet as possible before the experiment began. In the earlier work, both before and after exercise, one red and one white count and two blood films were made, all specimens being obtained by subcutaneous puncture. In later experiments at least two sets of counts and of films were made both before and after exercise; one set of specimens being obtained by subcutaneous puncture, the other set by withdrawing blood from a superficial leg vein. In some cases blood was also obtained by cardiac puncture.

After satisfactory specimens had been obtained the animal was exercised as vigorously as possible. A variety of modes of exercise was used. Among these were running in a treadmill, being towed by

a motorcycle, and running on a leash until the animal was well blown. Red and white counts and films, exactly comparable to those taken before exercise, were then secured, and the figures for all four observations compared. The results obtained in this way, as far as nucleated red cells are concerned, were remarkably uniform. Every dog observed, whose blood contained normoblasts before exercise, showed a slightly larger number of these cells after exercise. Some animals in whose blood no nucleated red cells were found before exercise displayed a few of these cells after vigorous muscular effort; other animals continued to show none. Typical examples of this result are shown in Table I.

The Effect of Exercise upon Hyperplastic Animals with Large Numbers of Nucleated Red Cells in the Capillary Blood.—Observations as to the effect of exercise upon normal dogs were followed by similar studies on a number of animals rendered hyperplastic by repeated hemorrhages of varying severity and frequency. During the course of development of hyperplasia the effect of exercise upon the animals was frequently tested. Exercise tests were made, at times a few hours after a hemorrhage, at other times days or even weeks after the last bleeding. Animals treated in this way pass through several phases in respect to the number of normoblasts which appear in the peripheral blood stream. Normal animals, showing a few normoblasts, may present a slight increase after the circulatory stirring up of exercise. In the 24 hours immediately following a large hemorrhage no significant increase in normoblasts occurs. Specimens taken before and at frequent intervals immediately after a hemorrhage either show no difference in nucleated red cell content, or show at best a slight increase after the hemorrhage, due in our opinion to a general stirring up of the circulation such as obtains in exercise.

But in the early stages of blood regeneration, evidenced by increasing red cell counts, normoblasts appear in larger numbers. Exercise increases the number of these cells in the capillary blood,—an increase practically in inverse proportion to the amount the animal has been allowed to move about before the specimens are taken. Later, as regeneration becomes complete, normoblasts largely disappear from the circulation, and at this stage few or none can be obtained by exercise.

It is evident, therefore, that exercise plus anemia will not bring

forth normoblasts, or else these cells would appear as a result of the exercise immediately after hemorrhage. Nor is exercise coupled with marrow hyperplasia capable of causing their appearance, for nucleated cells do not appear following exercise in the late stages of regen-

TABLE II.*

Hyperplastic Dogs Showing Large Numbers of Nucleated Red Cells.

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
	1916					
3	Oct. 7					Bled 350 cc.
Weight	" 16					" 175 "
12.5	" 20	2.58	4,440,000	15,800	316	
kilos.		3.01–3.16				Hard exercise in treadmill.
		3.21	4,412,000	16,600	896	
	Nov. 10					Bled 175 cc.
	" 17	2.10	3,928,000	50,700	659	
		2.15–2.26				Exercise, running in the hall.
		2.28	4,184,000	51,300	1,539	
4	Oct. 2					Bled 180 cc.
Weight	" 6	10.55	2,860,000	11,000	385	
7.5		10.59–11.11				Very moderate exercise.
kilos.		11.12	3,104,000	18,400	1,177	
	Oct. 9	4.05	4,024,000	15,100	1,721	
		4.08– 4.23				Moderate exercise in treadmill.
		4.30	4,312,000	16,800	2,318	
5	Oct. 21					Bled 360 cc.
Weight	" 22	9.40	2,736,000	17,300	138	
8.9		9.45–10.04				Hard exercise in treadmill.
kilos.		10.06	2,800,000	22,500	247	
	Oct. 25	9.30	2,976,000	34,200	684	
		9.31– 9.46				Exercise in treadmill.
		9.50	2,872,000	43,600	1,024	

* In bleeding animals, in order to secure as large a reduction of cells as was possible with safety, withdrawals of blood and injections of salt solution were alternated in the same operation. The figures given in the tables expressing the size of the hemorrhages represent, therefore, a mixture of blood and of recently injected salt solution.

TABLE II—*Concluded.*

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
	1917					
6						
Weight	Jan. 5					Bled 710 cc.
20.5	" 16					" 1,075 "
kilos.	" 17	2.25	3,048,000	36,700	1,321	From superficial vein.
		2.30	2,448,000	34,100	920	Subcutaneous puncture.
Average..			2,748,000	35,400	1,120	
		2.34– 2.46				Running out of doors; dog refused to run hard.
		2.55	2,078,000	38,000	1,596	From superficial vein.
		3.00	2,592,000	35,200	1,795	Subcutaneous puncture.
Average..			2,335,000	36,600	1,695	
	Feb. 2					Bled 1,160 cc.
	" 23					" 1,000 "
	Mar. 2	2.20	4,304,000	20,100	201	From superficial vein.
		2.25	4,280,000	20,800	145	Subcutaneous puncture.
Average..			4,292,000	20,450	173	
		2.27– 2.47				Hard running out of doors.
		2.50	4,528,000	20,300	1,096	From superficial vein.
		2.52	4,320,000	26,100	1,096	Subcutaneous puncture.
Average..			4,424,000	23,200	1,096	

eration when hyperplasia is extreme. Exercise gives more cells when cells are already present, and in this condition only.

The figures in Table II are taken from dogs in the early stages of the hyperplasia following hemorrhage. We have many more figures to the same effect but these given suffice to prove the point; namely, that animals, possessing in their capillary blood fairly large numbers of normoblasts before muscular effort, show still more of these cells after the general stirring up of exercise.

It seemed possible, at first, that the increase in nucleated red cells resulting from exercise was perhaps due to an output of cells from the marrow tissue, that the speeding up of the circulation occasioned by vigorous muscular effort had caused a real crisis of blasts, compar-

able perhaps to the blast crises of anemia. But further study convinced us that this interpretation was wrong. In experiments in which samples of blood were taken from different sources before exercising the animals, an unequal distribution of nucleated red cells was occasionally found. A specimen obtained by subcutaneous puncture, for example, would contain relatively few normoblasts while a second specimen taken from a superficial vein within a minute or two would show a much larger number of the cells. After exercise the second subcutaneous specimen would show a marked increase in the number of nucleated red cells, a result completely in accord with our former experiments. But the second vein specimen, on the contrary, would

TABLE III.

Effect of Exercise on Distribution of Nucleated Red Cells.

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
3	*1916*					
	Dec. 23	11.45	3,744,000	26,700	747	From superficial vein.
		11.50	3,616,000	27,100	336	Subcutaneous puncture.
Average..			3,680,000	26,900	541	
		11.55–12.17				Hard running out of doors.
		12.20	Lost.	24,600	565	From superficial vein.
		12.22	3,648,000	24,400	561	Subcutaneous puncture.
Average..			3,648,000	24,500	563	

show fewer nucleated red cells than before exercise. An average of the observations before and of those after exercise would give practically the same figure. The observations made on Dog 3, shown in Table III, illustrate this point.

This suggested that probably the increase of normoblasts in the capillary blood following exercise, which at first sight seemed possibly a real crisis of blasts extruded from the marrow tissue, was in reality merely the result of a general stirring up of the circulation and of a consequent more even distribution of blood through the peripheral area. Further experiments supported this idea. We found that any procedure calculated to cause a considerable stirring up of the circulation, such, for example, as hemorrhage and infusion, would im-

mediately increase the number of blasts in the peripheral blood, provided that any had been observed at the beginning. The suddenness of the increase in all these cases weighed in favor of the view that the newly observed cells had been hidden away in deep lying vessels, possibly in the veins of the marrow itself, and, as a result of the increased rate of circulation incident to the hemorrhage and infusion, had been driven out into the open.

As the experimental animals became more hyperplastic and developed refractory periods in relation to the extrusion of blasts, periods in which practically no nucleated red cells could be found in the peripheral blood either before or after exercise, we became convinced that exercise serves only to give a true picture of the nucleated red cells already in the circulation, and has no influence whatever on their extrusion from the marrow tissue.

The Effect of Exercise upon Hyperplastic Animals Showing Few or No Nucleated Red Cells in the Peripheral Blood Stream.—After a time many animals become adjusted to periodic bleedings and pass into periods when few or no nucleated red cells can be obtained by subcutaneous puncture, no matter how vigorous an effort is made to stir them out. These refractory periods have a definite relation to the process of regeneration after hemorrhage. In general they occur just after a hemorrhage when the red count is low, and again when regeneration is almost complete. If the animal has become adjusted to frequent bleedings and a particular hemorrhage is not a severe one, the refractory period may last throughout the entire process of regeneration. If, however, the hemorrhage is severe, the early refractory period is followed by a true formative crisis of blasts which lasts from 1 to several days. The height of this crisis varies with the severity of the hemorrhage and with the degree of ability to resist normoblast extrusion, which the animal may have developed.

During these two refractory periods, when few or no blasts are to be found in the peripheral blood, exercise will not bring them out. The figures given in Table IV are taken from two hyperplastic animals in refractory phases and will serve to demonstrate this point. The first figures in Table IV (Dog 5) represent a refractory period occurring after a hemorrhage when the red count was low. The second set of figures (Dog 7) are taken from a young dog whose

marrow was presumably hyperplastic at the beginning. When normal this animal showed no nucleated red cells before exercise and 123 per c. mm. after exercise. After one bleeding and regeneration to normal, he showed the completely refractory period recorded in Table IV. Throughout our entire experience with this animal he showed a remarkable resistance to the extrusion of blasts.

Table V gives an almost complete picture of a single dog passing through the various periods into which we have divided our exercise

TABLE IV.

Hyperplastic Dogs in Refractory Periods.

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
	1916					
5 Weight 8.9 kilos.	Oct. 21					Bled 360 cc. Animal showed many blasts during this period.
	Nov. 10					" 190 "
	Dec. 14					" 230 "
	" 19	4.15	3,456,000	23,100	0	
		4.20– 4.35				Exercise, running about building.
		4.38	3,128,000	24,600	0	
7 Weight 5 kilos.	Oct. 13					Bled 175 cc.
	" 24	9.40	5,064,000	19,500	0	
		9.42– 9.57				Hard exercise in treadmill.
		10.00	5,232,000	18,500	0	

experiments. Unfortunately this animal was not exercised before it was bled, so no figures can be given showing the reaction to exercise while yet normal. On January 5, the first experimental day, this animal was bled 710 cc. Before the bleeding two observations showed no normoblasts in the peripheral blood. After the hemorrhage, which reduced the red count from 5,696,000 to 3,831,000, eleven observations gave an average of 15 normoblasts per c. mm. This increase we believe to have been due to the stirring up of the circulation incident to the hemorrhage and infusion, and in this respect to be com-

TABLE V.

Effect of Exercise on Dog 6 during a Series of Hemorrhages and Recoveries.

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
	1917					
6 Weight 20.5 kilos.	Jan. 5	11.25–11.50	5,696,000	14,700	0	Average of two observations.
		12.00				Bled 710 cc.; infused 860 cc.
		12.10– 5.15	3,831,000	25,920	15	Average of eleven observations.
	Jan. 16					Bled 1,075 cc.
	" 17	2.25	3,048,000	36,700	1,321	From superficial vein.
		2.30	2,448,000	34,100	920	Subcutaneous puncture.
Average..			2,748,000	35,400	1,120	
		2.34– 2.46				Running out of doors; dog refused to run hard.
		2.55	2,708,000	38,000	1,596	From superficial vein.
		3.00	2,592,000	35,200	1,795	Subcutaneous puncture.
Average..			2,650,000	36,600	1,695	
	Feb. 2					Bled 1,160 cc.
	" 23					" 1,000 "
	Mar. 2	2.20	4,304,000	20,100	201	From superficial vein.
		2.25	4,280,000	20,800	145	Subcutaneous puncture.
Average..			4,292,000	20,450	173	
		2.27– 2.47				Hard running out of doors.
		2.50	4,528,000	20,300	1,096	From superficial vein.
		2.52	4,320,000	26,100	1,096	Subcutaneous puncture.
Average..			4,424,000	23,200	1,096	
	Mar. 8	2.50	4,992,000	24,600	24	From superficial vein.
		2.54	4,976,000	25,300	25	Subcutaneous puncture.
Average..			4,984,000	24,950	24	
		2.55– 3.10				Running out of doors; animal well blown.
		3.15	5,912,000	24,000	336	Subcutaneous puncture.
		3.17	5,880,000	23,200	116	From superficial vein.
Average..			5,896,000	23,600	226	
	Mar. 13	2.18	6,152,000	20,200	0	From superficial vein.
		2.21	5,824,000	22,200	22	Subcutaneous puncture.
Average..			5,988,000	21,350	11	

TABLE V—*Concluded.*

Dog.	Date.	Hour.	Erythrocytes per c.mm.	Leukocytes per c.mm.	Blasts per c.mm.	Remarks.
	1917					
6	Mar. 13	2.23– 2.38				Running out of doors.
		2.39	6,856,000	36,300	108	From superficial vein.
		2.40	6,616,000	32,200	96	Subcutaneous puncture.
Average..			6,736,000	34,250	102	
	Mar. 14					Bled 1,000 cc.
	" 17	8.35	4,072,000	27,860	55	From superficial vein.
		8.37	3,608,000	30,500	0	Subcutaneous puncture.
Average..			3,840,000	29,180	27	
		8.38– 8.55				Running out of doors.
		8.57	3,768,000	27,700	27	From superficial vein.
		8.58	3,448,000	26,500	53	Subcutaneous puncture.
Average..			3,608,000	27,100	40	

parable with an exercise increase. The figures on this animal for January 17, March 2, and March 8 show a typical reaction to exercise in the early stages of hyperplasia. The number of normoblasts in the animal's blood on these days was very high before exercise, and in each case was decidedly increased after exercise. It is interesting to note in this table the gradual development of an ability on the part of the animal to keep the nucleated red cells This tendency reaches its height, as far as this series of observations is concerned, on March 17, when the animal, starting with an average of 27 blasts per c. mm. before exercise, showed an average of 40 of these cells after exercise.

The Effect of Nerve Section.

In the early days of microscopic blood examinations, when the relative constancy in health of the red and of the white blood count was first appreciated and when the speed with which these counts could alter in disease became known, it was natural that investigators should look for nervous control of blood composition. Thus, Cohnstein and Zuntz (36) in 1888 examined the results of the complete vascular paralysis caused by spinal cord section at the level of the seventh cervical vertebra. They found that a great lowering of the red and of the white count followed this section, which was of course accompanied by complete vaso-

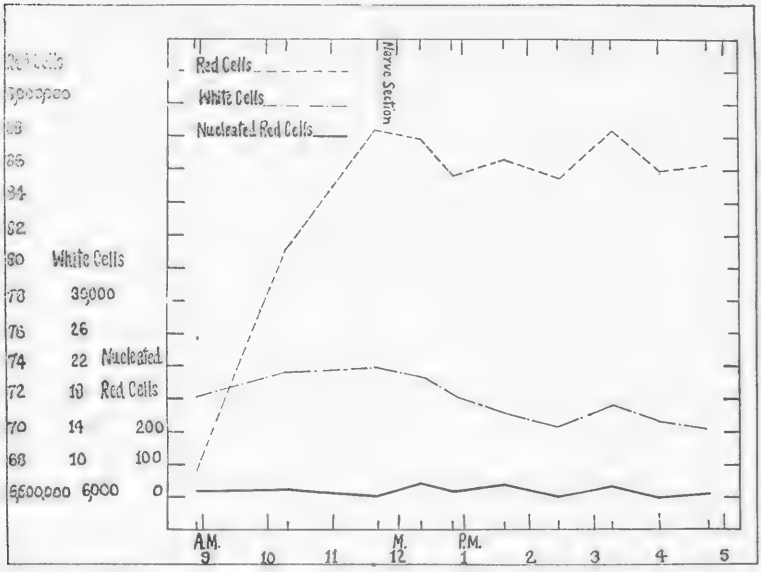
motor collapse. They attributed this blood dilution to transudation of tissue fluid into the vessels when their tone was destroyed. Malassez (37) in a brief note states that denervated parts usually give lowered red counts, but he presents no more than this brief statement.

It is obvious that such experiments as those of Cohnstein and Zuntz would not achieve the result demanded by our experiments, namely, increased flow through the marrow vessels, since in the collapse following complete vasomotor palsy the peripheral blood stream becomes very sluggish. It was therefore necessary to provide nerve sections which could be shown to have no effect on general blood pressure but which would at the same time open a large number of marrow vessels. We therefore cut the two sciatic nerves very high in the thighs and both brachial plexuses above the upper ends of the humeri. These sections result in vasomotor paralysis of the vessels in the long and the small bones of the four limbs. The operation in each experiment was carried out under sterile conditions. Blood examinations (erythrocyte, leukocyte, and normoblast counts) were made prior to surgical procedure. All operations were performed under morphine and cocaine anesthesia with brief administration of ether, if necessary, at the time of the nerve sections. Blood pressure tracings were made before nerve section and afterwards at intervals throughout the remainder of the day in order to follow the circulatory condition of the animal. The operation never gave shock. Following the nerve section we were able to maintain a normal blood pressure throughout the rest of the day while we followed at frequent intervals the cellular composition of the blood in order to see whether the increased blood flow through the bones enumerated caused egress of cells.

The results of the nerve section experiments were negative. There occurred either no change at all in the number of normoblasts present in the peripheral blood, or else so slight a change as to be within the limits of error of the method used in counting the cells.

Text-fig. 1 gives a graphic picture of a typical nerve section experiment performed on a normal dog (Dog 8). Three observations on this animal before nerve section, extending from 8.55 a.m. to 11.41 a.m. gave an average of 13 nucleated red cells per c. mm. of blood; seven observations after nerve section, extending over a period of 5

hours, gave an average of 21 nucleated red cells per c. mm.—an insignificant increase. The early rise in the erythrocyte count shown in this chart is a phenomenon, rather marked in this case, which we have often seen following morphinization. Before nerve section, however, the red count had reached a level which remained more or less constant throughout the afternoon.



TEXT-FIG. 1. Curve showing the absence of effect of section of nerves to the four limbs upon the red cell, the white cell, and the nucleated red cell counts in Dog 8. The ordinates represent cells per c. mm. of blood; the abscissæ represent time of day. The initial rise in the red cell count before nerve section is due to morphinization.

Fig. 1 gives the blood pressure record of the same animal during the course of the experiment. Tracing 1 was made shortly before section of the nerves. Tracing 2 was made before, during, and after nerve section, the time occupied by nerve section being indicated by two crosses (+ .... +) on the record. Subsequent tracings, 3 through 9, were made at intervals during the 5 hours following nerve

section. It will readily be seen that the animal maintained a normal blood pressure throughout the course of the experiment.

A similar experiment was performed on Dog 7 which had been bled and infused five times previously and whose marrow as seen at autopsy showed extreme red cell hyperplasia. Two observations on this animal before nerve section gave an average of 41 nucleated red cells per c. mm.; eleven observations, during a period of about 5 hours following the operation, gave an average of 47 nucleated red cells per c. mm. In this animal $3\frac{1}{2}$ hours after section of the nerves the effect of general asphyxia upon normoblast extrusion was tested by repeated clampings of the trachea. This procedure caused each time a marked rise in blood pressure but had no effect upon the appearance of nucleated red cells in the peripheral blood.

With the exception of one animal (Dog 9), in which the small but decided increase after nerve section of from 645 (average of three observations) to 908 (average of seven observations) normoblasts per c. mm. was obtained, all nerve section experiments gave negative results.

CONCLUSIONS.

1. Increase in circulatory rate caused by hard exercise has no power to dislocate nucleated red cells from the bone marrow either in normal, in anemic, in hyperplastic, or in anemic and hyperplastic animals.

2. In anemic and hyperplastic animals pseudocrises of nucleated red cells can be produced at certain periods by hard exercise, but careful analysis leads inevitably to the conclusion that the increase in these cells is merely a more accurate expression of circulatory content at the time of the procedure.

3. Section of the vasomotor nerves to the four limbs with consequent dilatation of the marrow vessels and increased blood flow through the tissue does not result in the freeing of nucleated red cells from the bone marrow.

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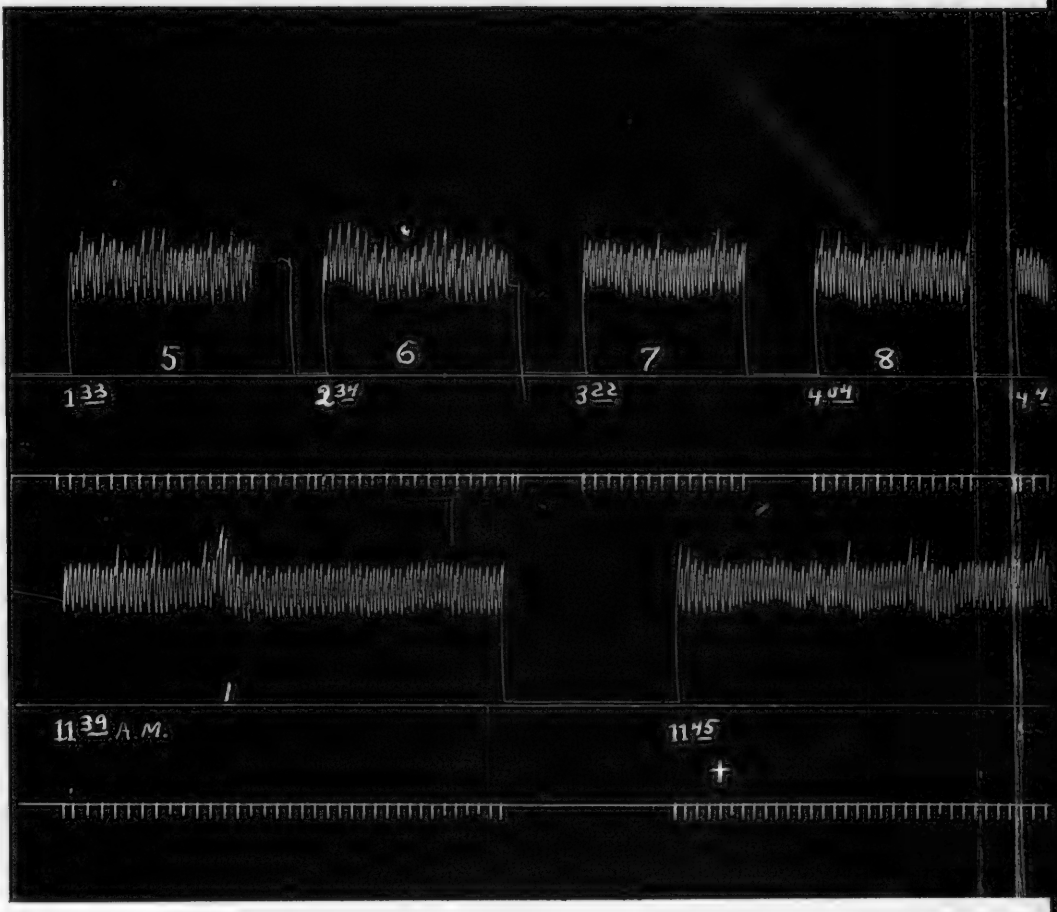
EXPLANATION OF PLATE 7.

FIG. 1. Graphic record of the arterial blood pressure of Dog 8 taken at intervals during a period of 5 hours preceding, during, and following section of nerves to the four limbs. Nerve section, performed during the recording of tracing 2, caused no immediate or subsequent fall in general arterial pressure.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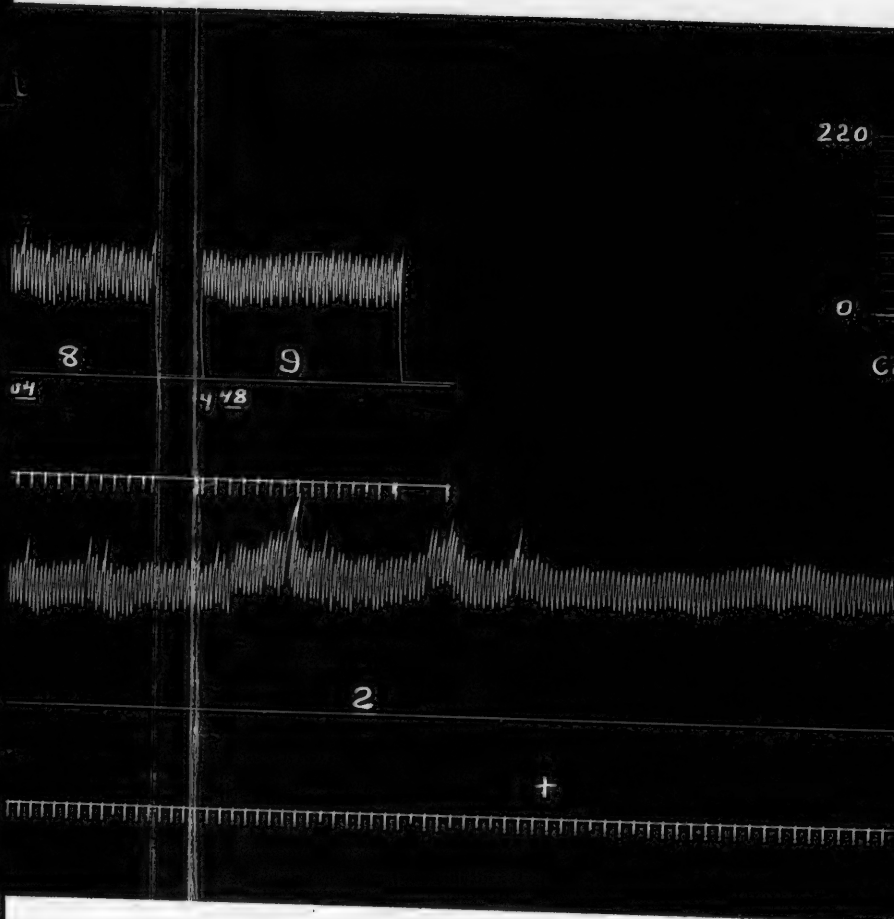
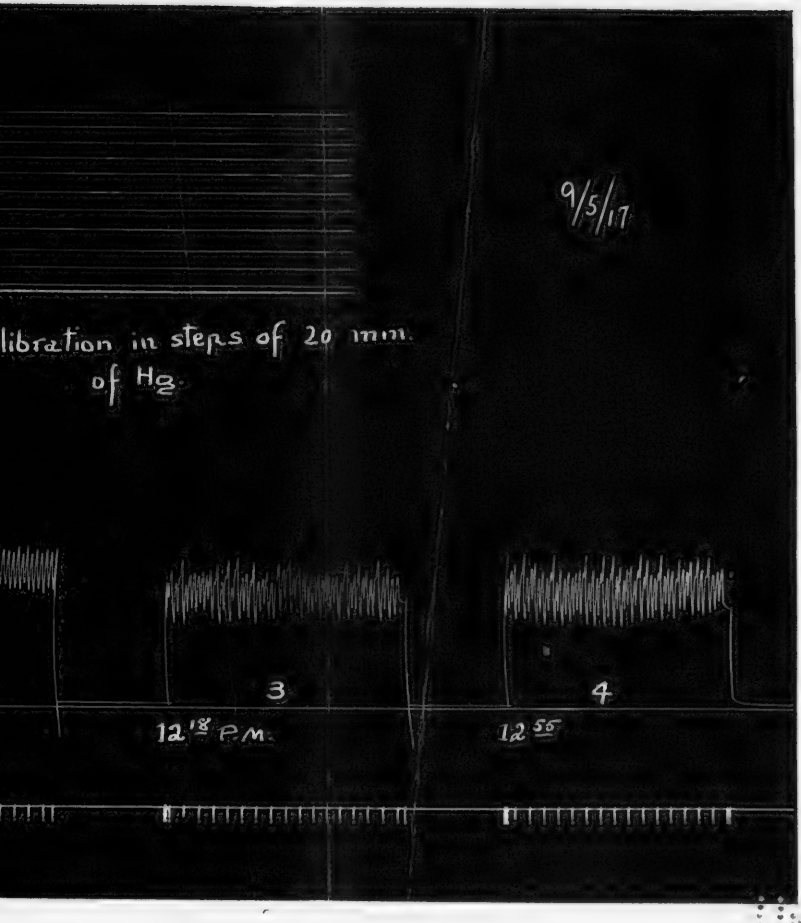


FIG. 1.



(Drinker, Drinker, and Kreutzmann: Nucleated red blood corpuscles. I.)

SKIN FERMENTS.

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(Received for publication, September 7, 1917.)

During the past few years, particularly in connection with observations on the luetin reaction, the typhoidin reaction, and related bacterial skin tests, as well as with certain skin tests made with a variety of proteins in clinical conditions apparently dependent on a sensitized state of the patient (asthma, urticaria, etc.), greater interest has been stimulated in the study of the possible mechanism of these reactions and their relation to antibody as well as ferment concentration of the reacting tissues.

Kolmer (1) has reviewed the subject in a recent paper. He calls attention to the fact that, aside from the sharply specific reactions, certain non-specific and as yet unknown factors play a part in some of the skin reactions. The work that has followed the observations of Sherrick (2) concerning the part the iodides play in influencing skin reactivity has emphasized this point of view. With these considerations in mind we have undertaken a study of the enzymes of human skin and of skin from several of the common laboratory animals.

Phylogenetically we might expect that the skin would elaborate ferments to a greater degree than most other tissues, since the ectoplasm of the cell (and the differentiated ectoderm) apart from its protective function is that portion of the cell brought into relation with the surrounding medium and therefore intimately associated with any secretory mechanism designed to alter food particles through extracellular digestion. Of course, with the continued differentiation into a protective structure we may assume that the ferment secretory activity would be repressed. As far as the literature is available to us we have found but few references to work dealing with skin ferments.

Material.—The skin material was prepared as follows: The skin was stripped from the underlying tissue from freshly killed animals, and from fresh autopsy material in the case of the human skins. The animals were not exsanguinated, so that the normal amount of blood remained in the corium. With a razor as much of the connective tissue of the corium as possible was then removed, leaving the flaps practically free from connective tissue. The skin was next cut into small bits with sharp scissors, spread on a glass plate, and rapidly dried in an air current at low temperature (25°C.). The dried material was then placed in a desiccator over night and ground the following morning. The powdered skin was put through a 40 mesh sieve. The dried powder was made up as a 0.5 per cent suspension in isotonic salt solution for use.

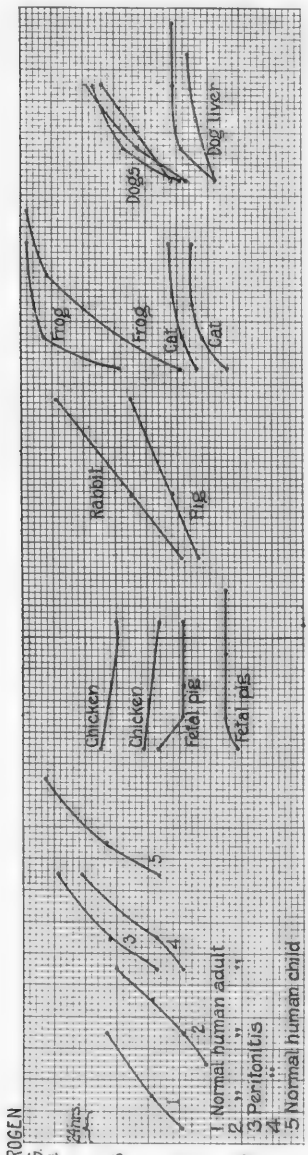
Inasmuch as the antigens ordinarily employed in skin reactions are largely protein in character, we have endeavored to study the proteolytic processes of the skin in greater detail, including (*a*) the simple autolysis of the skin (without altering the reaction); (*b*) digestion of a foreign protein (casein); (*c*) the relative resistance of the skin to tryptic digestion; (*d*) the peptidase content of the skin.

Autolysis of the Skin.—The skin suspensions were autolyzed for several days under toluene at 45°C. At definite times, 24, 48, 72 hours, etc., 2 cc. samples were withdrawn and the amount of non-coagulable nitrogen was determined. The relative rate of autolysis is illustrated in Text-fig. 1.

The human skins all autolyzed at practically the same rate and evidently to an extent equal to that of the animal skins. On the other hand, the skin of the young animals (fetal pig, young chicken) gave no evidence of autolysis, while of the skins of the adult animals that from the frog autolyzed to the greatest extent. For purposes of comparison the autolysis of the liver, dried with its contained blood, is shown in the last curve of Text-fig. 1.

Protease.—In order to study the possible liberation of proteases from the skin two experiments were made. In the first, 1 cc. each of the skin emulsions was incubated 48 hours with 2 cc. of a 1 per cent casein solution made slightly alkaline to phenolphthalein with sodium carbonate.

No alkaline active protease was demonstrable by this means. In



TEXT-FIG. 1. The relative rate of autolysis of skin suspensions from human subjects and animals.

the following experiment the casein solution was prepared slightly acid to phenolphthalein, and in this experiment the digestion of the casein proceeded as indicated in the following table:

	Nitrogen. mg.		Nitrogen. mg.
Liver	0.57	Rabbit	0.20
Normal human	0.3	Chicken	0.11
" dog	0.29	Pig	0.00
" "	0.11	Cat	0.00
" "	0.09	Frog	0.00

Resistance to Tryptic Digestion.—2 cc. of the skin suspension were incubated for 4 hours with $1\frac{1}{2}$ trypsin units. 1 unit digests 2 cc. of a 1 per cent casein solution in 1 hour. For purposes of control similar tubes were prepared and immediately boiled. The amount of the increase in non-coagulable nitrogen as a result of the digestion is shown in the following table:

	Nitrogen. mg.		Nitrogen. mg.
Dog	0.23	Pig	0.00
"	0.07	Fetal pig	0.00
"	0.05	Frog	0.00
Rabbit	0.17	Cat	0.00
Chicken	0.02	Human (5 specimens)	0.00
Pig	0.05		

Peptidase.—Peptidase was determined by the usual method of digesting Witte's peptone, glycyltryptophane not being available, and determining the formation of free tryptophane with bromine water. In the tests an equal volume of the skin suspension was mixed with a 10 per cent solution of the peptone and incubated for 72 hours, in some instances for 120 hours. The following table illustrates the relative intensity of the digestive effect of the skin.

Fetal pig	++++	All other human skins	0
Chicken	++	Dog	0
Cat	+	Rabbit	0
Guinea pig	Tr.	Frog	0
Human (normal boy)	"	Pig	0

Lipases.—Porter (3) in his study of the distribution of the lipases of the body has noted the relative richness of the skin tissues in the true lipases and cholesterolases. In view of the function of the skin

in the elaboration of the sebaceous secretion this is to be expected. Porter calls attention to the possible part these ferments play in the relative resistance of the skin to tuberculosis as well as to the bacteriological evidence that the tubercle bacilli in lupus are not only few in number but not infrequently lose their acid-fast character.

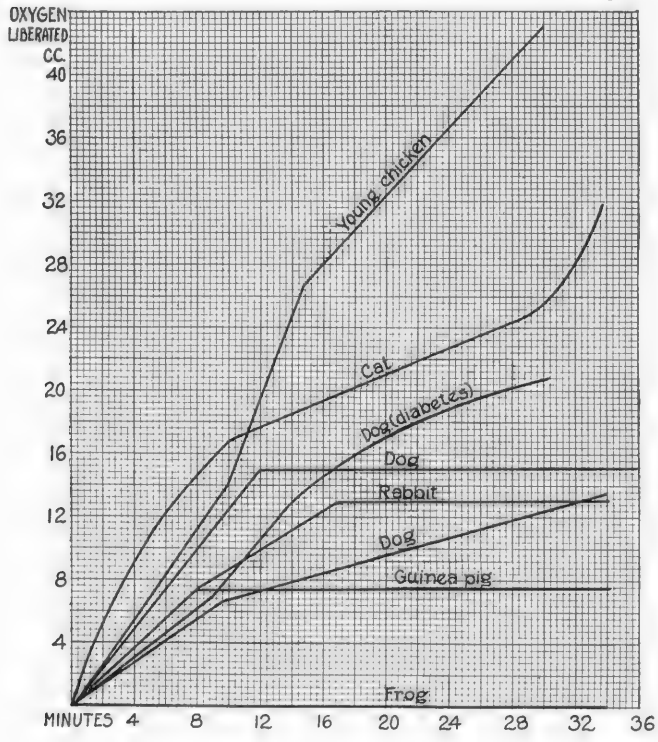
The lipolytic determinations were made with the following substrates: ethyl butyrate, 10 per cent butter fat emulsion, and 10 per cent olive oil emulsion. One experiment was made in which the skin powder was added directly to the olive oil without the addition of water. For purposes of relative orientation we have used a preparation of dog liver, prepared in a similar manner to the skin, with each substrate.

Material.	Substrate.			
	Ethyl butyrate.	Butter fat.	Olive oil emulsion.	Olive oil.
	cc.	*cc.*	*cc.*	*cc.*
Liver	1.5	0.74	3.5	6.0
Pig skin, white	0.5			
" " pigmented	0.37			
Fetal pig skin	0.75			
Human skin	0.1			
" "	0.15	0.6	0.45	0.22
" "	0.1	0.95	0.47	0.14
" "	0.15	0.66	0.3	0.12
" "	0.0			
Chicken "	0.23	2.00	0.5	0.20
Cat	0.23	0.65	0.47	0.15
Frog	0.25	0.59		
Dog	0.25	0.50	0.86	0.15
"	0.20			
"	0.20			
Rabbit	0.20	0.55	0.54	0.35
Guinea pig		0.6	0.75	0.15

In all cases the incubation was for 24 hours at 45°C. and the resulting acidity has been expressed in cc. of 0.05 N sodium hydroxide.

Catalase.—The catalase content of the skin was determined by the ordinary volumetric method and the results are expressed in the accompanying charts in cc. of oxygen liberated by 100 mg. of the dried skin from 50 cc. of a 50 per cent peroxide solution.

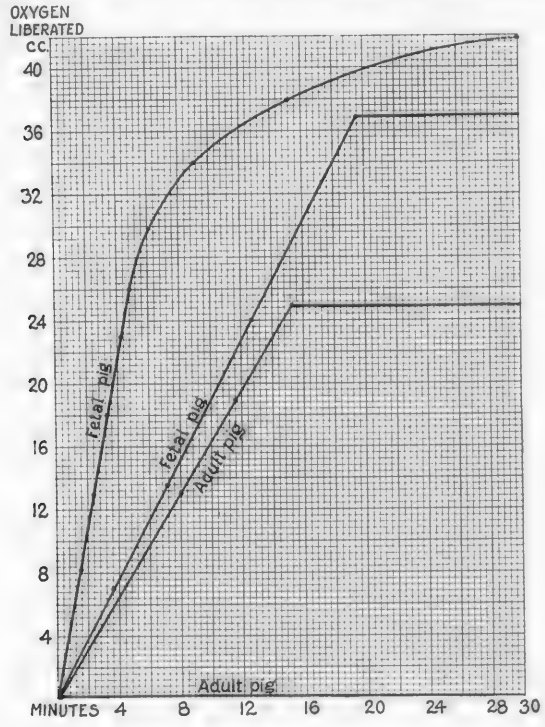
It will be observed that there is considerable variation in the skins, from the 43 cc. liberated in 30 minutes by the young chicken skin to none in the case of frog skin. The skin of the depancreatized dog retained its catalase content despite the fact that the content of the



TEXT-FIG. 2. Determination of the catalase content of the skin of different animals.

other tissues (liver and heart muscle), as determined by Kennedy and Burge (4), is decidedly reduced (Text-fig. 2). A comparison of the fetal and adult skin, made on the skin of pigs, would indicate that the fetal skin contained considerably more catalase than that of the adult (Text-fig. 3).

For purposes of orientation we have studied two normal human skins from accident cases and two skins from pathological postmortem material, one acute alcoholism, the other pregnancy, abortion, and peritonitis. The normal skins are evidently higher in catalase content than the skins from the pathological cases, but without an

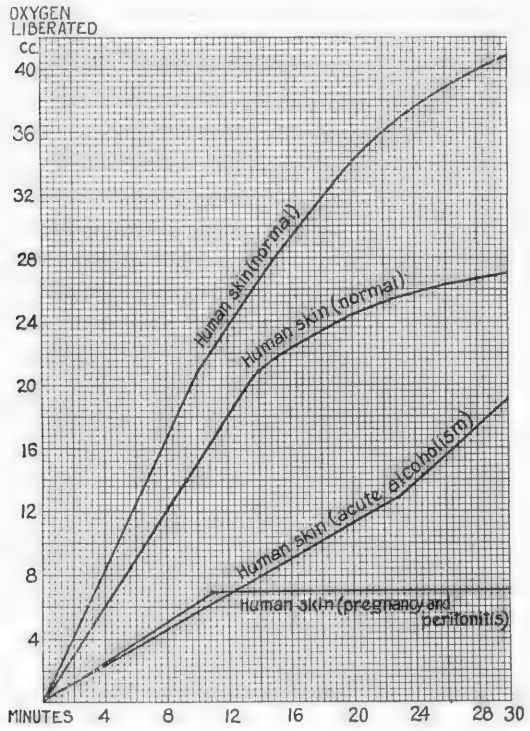


TEXT-FIG. 3. Comparison of the catalase content of the fetal and adult skin.

extended series of observations it would be impossible to draw definite conclusions concerning this point (Text-fig. 4).

Diastatic Ferments.—Progressively diminishing amounts of the skin suspension were incubated for 24 hours with 2 cc. of a 1 per cent solution of starch, and the digestive activity was titrated. The relative diastatic activity of the tissues was found to be as follows:

	$D \frac{37°}{24\ hrs.}$		$D \frac{37°}{24\ hrs.}$
Fetal pig	32	Cat	12
Chicken	16	Human	4
Liver	16	Guinea pig	4
Boy	12	Pig	4
Frog	12	Rabbit	2



TEXT-FIG. 4. Catalase content of two normal skins and two skins from pathological postmortem material, one acute alcoholism, the other pregnancy, abortion, and peritonitis.

DISCUSSION AND SUMMARY.

That a definite change in the reactivity of the skin takes place during the transition from infancy to childhood has been repeatedly noted. Rolly (5), working with a variety of bacterial toxins, found that infants did not react, but that reactions occurred with advancing

age. Similarly Tezner (6), using Witte's peptone, colon bacilli, and tuberculin observed that at about the period that the skin of children became increasingly sensitive to tuberculin, a corresponding reactivity was manifest toward the other antigens. It seems possible to us that this phenomenon is related to the alteration in the ferments of the skin that takes place during the transition period. Thus while the protease of the adult type of skin causes a definite autolysis, that of the fetal skin does not, the synthetic potential evidently predominating. Of greater significance seems to be the fact that the skin of the young animal contains peptidase to a considerable amount, while the adult skin seems to be without this ferment activity.

If we assume that the disintegration of the native protein of the antigen is essential in eliciting certain skin tests, it would seem probable that the splitting would be less apt to take place in the young skin in which the lytic effects of the ferments are relatively suppressed. On the other hand, the young skin, containing peptidase, would be able to digest rapidly any antigen consisting of partially hydrolyzed proteins—as tuberculin—and in this way detoxicate and remove the noxious material. From these considerations the undoubted alteration of the skin reactivity taking place after infancy seems definitely correlated with changes in the proteolytic ferments. In how far they enter into and alter the specific reactions cannot be determined from the limited data so far available.

Of the other ferments, the lipases, as pointed out by Porter, are possibly of importance in the resistance of the skin to tuberculous infection. It will be observed from the table that the activity of these skin ferments is less manifest in their action as esterases, on ethyl butyrate, than on the neutral fats used, oleic oil and butter fat, in this way differing from the activity of the serum. The relative resistance of the skin to tuberculous infection can, however, hardly be due to the activity of the lipase alone, for it must be kept in mind that the lepra bacillus, also rich in lipoids, is enabled to proliferate well within the epithelial tissues.

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INTRAVENOUS SEROTHERAPY OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).*

By RYOKICHI INADA, M.D., YUTAKA IDO, M.D., ROKURO HOKI, M.D., HIROSHI ITO, M.D., AND HIDETSUNE WANI, M.D.

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(Received for publication, September 4, 1917.)

We have already reported[1] the results of our studies with subcutaneous injections of immune serum in spirochætosis icterohæmorrhagica. In the present article are presented the results of injections made by the intravenous method, which has in the main been employed in the serotherapy of Weil's disease since May, 1916. The number of patients treated in this manner in our clinic from May, 1916, to November, 1916, was 41. We found that the intravenous injection of immune serum far exceeds in potency the subcutaneous injection. An explanation of this finding may be sought in the fact that the therapeutic effect of serum injected directly into the blood stream is 500 times more potent than when it enters the organism by the subcutaneous route (Berghaus).

Experiments to show the comparative effects of the subcutaneous and intravenous administration of serum in Weil's disease were undertaken with seven rabbits, the animals receiving 0.5 to 1 cc. of immune serum per kilo of body weight. At various intervals the blood of the animals was examined for spirocheticidal and spirochetolytic immune bodies. The blood was taken from the ear vein on the side opposite the one in which the intravenous injection was made. The results of these experiments are given in Table I.

The immune bodies could be demonstrated as complete 5 minutes after the intravenous injection of 0.5 cc. of serum per kilo of body weight, while with the subcutaneous method the immune bodies

* Presented at the meeting of the Japanese Hygiene Association, April, 1917.

[1] Inada, R., Ido, Y., Hoki, R., Ito, H., and Wani, H., *J. Exp. Med.*, 1916, xxiv, 485.

could be detected only 8 hours after the injection and were then not completely absorbed. This passive immunity continued for 3 or 4 days. Complete immunity could not be obtained in man with the use of the small dose injected subcutaneously, but when larger doses

TABLE I.

Immune Bodies in Rabbits Infected with the Blood of Patients Having Weil's Disease.

Animal No.	Method of injection.	Amount injected.	Amount of serum per kg.	Body weight.	Pfeiffer's phenomenon.															
					Before serum injection.	After serum injection.														
						Min.			Hrs.					Days.						
						5	15	30	1	3	5	8	12	24	2	3	4	5	6	7	8
		cc.	*cc.*	*gm.*																	
1	Intravenous.	1.25	0.5	2,500	−		+	+	+							+	+		−		
2	"	2.5	1.0	2,500	−	+		+	+							+	+		−		−
3	Subcutaneous.	2.1	1.0	2,100	−	−		−	−					+	+	+	±		−		
4	"	1.7	0.5	3,400	−			−	−	−	−	−	±	±			±	−			
5	"	1.55	0.5	3,100	−				−	−	−	±	±	±	±	±	±	−			
6	"	3.1	1.0	3,100	−				−	−	−	−	±	±	±	±	±	−			
7	Intravenous.	1.45	0.5	2,900	−	+			+							+	−	−			

Titer of Serum Employed in the Experiments.

Pfeiffer's phenomenon.		Course of disease.	Remarks.
Amount of serum.	No. of spirochetes.		
cc.			
0.001	Liver emulsion (10 spirochetes per field) 1 cc.	Died 5th day of icterus.	Control died 4th day of icterus.
0.005	" " "	" 16th day without icterus. Autopsy negative.	" " "

− indicates no immune bodies; + immune bodies complete; ± immune bodies incomplete.

were given a complete passive immunity was attained also with the subcutaneous method.

The sera used were obtained from horse's blood as follows: No. 2, February 26, 1916; No. 6, August 7; No. 7, August 7; and No. 8, August 19. One of the horses was immunized from the beginning

with a living culture of *Spirochæta icterohæmorrhagiæ;* four others received first an inoculation of the killed culture, followed later by the living culture. The latter procedure can be more readily carried out. The animal receiving the living culture showed on the 7th day a temperature of 39°C. All the horses were immunized by a uniform procedure.

Titer.—The titer of the immune serum was determined by testing

TABLE II.

Titer of Serum.

No. of serum.	Pfeiffer's phenomenon.		Course of disease.	Remarks.
	Amount of serum.	No. of spirochetes.		
	cc.			
2	0.005	Liver emulsion (10 per field) 1 cc.	Died 12th day of icterus.	Control died 4th day of icterus.
	0.01	" " "	Died 18th day without icterus. Autopsy negative.	" " "
6	0.01	Liver emulsion (10 per field) 1 cc.	Well for more than a month.	Control died 4th day of icterus.
7	0.01	Liver emulsion (10 per field) 1 cc.	Died 10th day of icterus.	Control died 4th day of icterus.
	0.03	" " "	Well for more than a month.	" " "
8	0.01	Liver emulsion (10 per field) 1 cc.	Well for more than a month.	Control died 4th day of icterus.

the quantity which would protect a guinea pig weighing 200 gm. against infection from 1 cc. of pure culture containing 10 spirochetes per field (Leitz $\frac{1}{12}$ oil immersion, ocular 3). The spirocheticidal titer of the serum was 0.01 and 0.03 cc.; it has been found that a titer of 0.01 cc. suffices for the serotherapy of Weil's disease. Table II shows the spirocheticidal and spirochetolytic effects of the immune sera.

The experiments were made with sterile sera without preservative (phenol). As shown in Table II, Sera 2, 6, and 8 were more effective than No. 7.

Dose.—As a rule, 60 cc. of serum were injected intravenously, irrespective of the severity of illness, sometimes the entire quantity being given within 24 hours; or 40 cc. in a day, and the remaining 20 cc. the following day; or 20 cc. daily for 3 successive days. We cannot at present state definitely which procedure is to be preferred, and in order to determine this point, it will be necessary to make observations on a larger number of patients than is here presented. In any case, it is clear that the dose depends upon the severity of the disease. The entire amount of 60 cc. is not needed in the treatment of the less severe cases, although this quantity is always put up for an individual dose. In milder cases, the injection of from 20 to 40 cc. of serum is sufficient.

As is the usual custom, the serum was introduced into the vein of the arm. Previous to the injection 2 to 3 cc. of serum were injected subcutaneously into the chest or the thigh of the patient. The arm injection was then made 2 or 3 hours later, the serum being permitted to run in slowly, allowing 5 minutes for the introduction of 20 cc. The serum should be warmed to body temperature before use.

Day of Injection.—This has an important bearing on the course of the disease. As already emphasized, the action of the immune serum is spirocheticidal and spirochetolytic. The best results are obtained when the injection is made at an early stage of the disease. The question now arises: Up to what day of illness may the injections be continued with success? This period can be determined by studying (1) the infectivity of the patient's blood for guinea pigs, (2) the distribution of the spirochetes in the organs at various stages, and (3) from clinical observations of patients receiving the intravenous treatment. Table III gives the infectivity of the blood on various days of the disease. It is based on 42 cases treated recently, and 69 older cases. It will be observed that the infectivity diminishes gradually in the course of the disease. On the basis of these findings it would seem that the intravenous injection is effective up to the 5th day.

The investigations of Kaneko and Okuda concerning the distribution of spirochetes in the organs in Weil's disease indicated that the liver harbors few or wholly degenerated forms on the 7th day. In two patients on whom a post-

mortem examination was made on the 6th day, spirochetes were found in moderate numbers, though not so numerously as in the guinea pig. According to this, as already stated, we may expect the intravenous injection to be successful up to the 5th day. From our clinical observations, an undoubted effect can be obtained up to the 6th day of illness. We had few cases of this kind, however.

Four patients received the intravenous injection on the 3rd day, 9 on the 4th, 6 on the 5th, and 5 on the 6th day. Patients admitted at a later stage received an initial intravenous injection, not fol-

TABLE III.

Infection Experiments on Guinea Pigs with the Blood of Patients Having Weil's Disease.

Day of illness.	Cases previously examined.	Positive cases.	Negative cases.	Per cent positive.	Recently examined cases.	Positive cases.	Negative cases.	Per cent positive.	Total no. of cases.	Positive cases.	Negative cases.	Per cent positive.
2	4	4	0	100.0	0	0	0	0	4	4	0	100.0
3	10	10	0	100.0	4	4	0	100.0	14	14	0	100.0
4	13	13	0	100.0	9	9	0	100.0	22	22	0	100.0
5	12	11	1	91.7	6	5	1	83.3	18	16	2	88.9
6	14	12	2	85.7	5	2	3	40.0	19	14	5	73.7
7	8	4	4	50.0	7	3	4	42.9	15	7	8	46.7
8	4	0	4	0	3	1	2	33.3	7	1	6	14.3
9	1	1	0	100.0	5	0	5	0	6	1	5	16.7
10	0				1	0	1	0	1	0	1	0
11	0				1	0	1	0	1	0	1	0
12	1	0	1	0	1	0	1	0	2	0	2	0
18	1	0	1	0					1	0	1	0
19	1	0	1	0					1	0	1	0
Total No. of cases...	69				42				111			

lowed by others. 12 of the patients showed no icterus on admission, although 4 had a slightly yellowish pigmentation of the conjunctivæ. Of these 12 patients, 6 failed to develop icterus in the further course of the disease. Classified according to the severity of illness, we treated 6 slightly ill, including atypical cases, 9 moderately ill, and 26 severe cases. At the time that the intravenous treatment was being administered, we had in the clinic 11 other patients who re-

ceived no serum treatment; *i.e.*, 4 atypical cases, 1 slightly ill, and 6 moderately ill. There were no severe cases among them. It will be noted that the serum was administered in the main only to those who were severely or moderately ill.

Age.—We treated 9 patients between the ages of 16 and 20 years, 9 between 21 and 30, 6 between 31 and 40, 10 between 41 and 50, 4 between 51 and 60, and 3 who were over 60 years of age. We desire to add here that our patients, in addition to the serotherapy, received the benefit of the other usual methods of treatment in Weil's disease; *i.e.*, the intravenous injection of Ringer's solution and the subcutaneous administration of camphor and cocaine.

Spirocheticidal and Spirochetolytic Effect of the Immune Serum Injected Intravenously.

The spirochetolytic and spirocheticidal effects of the serum can be most readily shown when the spirochetes are demonstrable in the peripheral blood by dark-field illumination. In that case no infection experiments need be made. Up to the present time, however, we have observed this to be true in only 2 out of 100 cases. These 2 cases are discussed below.

Case 1.—M., male; age 47 years. The patient was admitted to the clinic on November 4, 1916, on the 3rd day of illness. This was a severe case. The blood showed by dark-field illumination 1 spirochete in from 70 to 140 fields. 1 hour after the intravenous injection of Serum 6, no spirochetes could be detected in two preparations. Blood was then withdrawn from the arm vein, and two guinea pigs received each an intraperitoneal injection of 1 cc. of the patient's blood. The animals became ill and showed typical symptoms 1 or 2 days later than the control animals, which had received blood withdrawn prior to the injection of serum. Although no spirochetes were found in two preparations, the blood was still infectious.

Case 5.—N., male; age 65 years. He became suddenly ill on May 1, 1916, and was admitted to the clinic on May 4, the 4th day of illness. We were able to demonstrate numerous *Spirochæta icterohæmorrhagiæ* in the blood by dark-field illumination in 14 to 16 specimens of 65 to 70 fields each. As a rule, spirochetes are not readily found in fresh preparations. The findings of spirochetes before and after serum injections were as follows:

2 p.m. Spirochetes in the blood, 14 to 16 in 65 to 70 fields.
3 p.m. 17 cc. of Serum 2 injected subcutaneously.
6 p.m. 14 to 16 spirochetes in 65 to 70 fields.

7 p.m. 10 to 16 spirochetes in 65 to 70 fields.
8 p.m. 20 cc. of Serum 2 injected intravenously.
10 p.m. No spirochetes in 2 preparations.
12 m. No spirochetes in 3 preparations.
1 a.m. 2 cc. of patient's blood injected intraperitoneally into a guinea pig, which remained well (May 14).
1.30 a.m. No spirochetes in 1 preparation.
8.30 a.m. 3 cc. of patient's blood injected intraperitoneally into a guinea pig, which remained well.

2 hours after the intravenous injection of serum in Case 5, we were unable to find any microorganisms. This demonstration of the spirocheticidal and spirochetolytic action of the immune serum in man exactly parallels the results obtained with guinea pigs. The patient died, however, at 5.30 p.m. on the following day. But the unexpected results obtained in this case induced us in later cases to employ only the intravenous injection method.

The experiments demonstrate clearly the spirocheticidal and spirochetolytic action of the immune serum, which is capable of destroying the spirochetes in the blood stream within a short period of time.

In the other thirty-nine cases spirochetes could not be readily demonstrated in the blood by dark-field illumination. Hence we conducted infection experiments before and after serum injection, similar to those made with the subcutaneous serum cases (Table IV). Table V gives the results of the infection experiments, the serum administered, and the day of illness when the blood was taken. We have omitted from the table the cases which were negative with the use of blood drawn before serum was injected.

The infection experiments were all negative following the injection of 20 cc. of Serum 2. With Serum 6, only one experiment was positive. In this instance the animal was inoculated 1 hour after the injection of the patient. It appears that Sera 2 and 6 possessed markedly potent spirocheticidal and spirochetolytic properties, while No. 7 was less effective; the titer of the latter serum was 0.03 cc. Although the infection experiments were positive with blood taken after the injection of Serum 7, the animals became typically ill much later than the animals receiving blood drawn before the injection. The difference in the duration of the life of the animals in the two groups was from 3 to 7 days, as in Cases 10, 11, 12, 13, 19, and 25.

TABLE IV.

Infection Experiments with Patients' Blood Withdrawn before and after Intravenous Injection of Serum.

Case No.	Sex.	Day of admission.	Day of illness just before serum injection.	Results on guinea pigs.				Amount of serum injected.	No. of serum.	Time between serum injection and withdrawal of blood.	Day of illness after serum injection.
				Before serum injection.		After serum injection.					
				No.	Result.	No.	Result.				
		1916						*cc.*		*hrs.*	
1	Male.	Nov. 4	3	2	+(7 days). +(8 ").	1	+(9 days).	20	6	1	3
						1	+(9 days).	20		2	3
2	Male.	Sept. 8	3	2	+	2	–	20	7	3	3
3	Male.	Sept. 15	3	2	+	2	–	20	7	3	3
4	Male.	Sept. 22	3	2	+(9 days).	2	+(9 days).	20	7	5	3
5	Male.	May 4	4	1	+	1	–	40	2	5	4
6	Female.	May 30	4	1	+	1	–	20	2	3	4
7	Female.	June 20	4	1	+	1	–	20	2	10	5
8	Male.	Aug. 31	4	2	+	2	–	20	2	3	4
9	Male.	Sept. 22	4	2	+	2	–	20	6	3	4
10	Male.	Sept. 8	4	2	1+(6 days). 1+(8 ").	2	+(13 days).	20	7	3	4
11	Female.	Sept. 19	4	2	1+(6 days). 1+(8 ").	2	+(11 days).	20	7	3	4
						2	–	20		18	5

12	Male.	Sept. 10	4	2	+(7 days).	2	+(12 days).	20	7	3	4
13	Male.	Nov. 19	4	1	+(6 days).	1	+(11 days).	20	8	3	4
14	Male.	Aug. 28	5	2	1+ 1−	2	−	20	2	3	5
15	Male.	Oct. 20	5	2	−	2	−	20	6	3	5
16	Male.	Nov. 12	5	1	+	1	−	20	6	3	5
17	Male.	Nov. 20	5	1	+	1	−	20	6	3	5
18	Male.	Nov. 26	5	1	+	1	−	20	6	3	5
19	Male.	Sept. 6	5	2	+(7 days).	2	1+(12 days). 1+(13 ").	20	7	3	5
20	Female.	May 28	6	1	−	1	−	20	2	4	6
21	Female.	June 26	6	1	−	1	−	20	2	3	6
22	Female.	Aug. 23	6	1	?	1	?	20	2	3	6
						1	−	40		9	7
23	Male.	Oct. 16	6	2	1− 1?	2	−	20	6	3	6
24	Female.	Oct. 26	6	2	−	2	−	20	6	3	6
25	Male.	Sept. 10	6	2	+(8 days).	2	+(13 days).	20	7	3	6

TABLE IV—*Concluded.*

Case No.	Sex.	Day of admission.	Day of illness just before serum injection.	Results on guinea pigs.				Amount of serum injected.	No. of serum.	Time between serum injection and withdrawal of blood.	Day of illness after serum injection.
				Before serum injection.		After serum injection.					
				No.	Result.	No.	Result.				
		1916						*cc.*		*hrs.*	
26	Female.	Nov. 19	6	1	+	1	—	20	8	3	6
27	Male.	July 25	7	1	+	1	—	60	2	11	8
28	Female.	June 24	7	1	+	1	—	20	2	3	7
29	Male.	July 6	7	1	—	1	—	20	2	3	7
30	Female.	Aug. 31	7	2	—	2	—	20	2	3	7
31	Male.	Sept. 2	7	1	—	2	—	20	2	3	7
32	Male.	Oct. 12	7	2	1+ 1—	2	—	20	6	3	7
33	Male.	Sept. 15	7	1	—	1	—	20	7	3	7
34	Male.	May 20	8	1	—	1	—	20	2	8	8
35	Female.	Nov. 15	8	1	—	1	—	20	7	3	8
36	Female.	Nov. 22	10	1	—	1	—	20	2	3	10
37	Male.	Sept. 15	11	2	—	2	—	20	6	3	11

Cases 1 and 4 are exceptions. It is evident that the serum was potent after 3 hours, although it was incapable in this short period of time of destroying completely the spirochetes contained in the blood. This is true also of Serum 8.

The time elapsing between the injection of serum and the withdrawal of blood varied from 1 to 18 hours, the usual interval being 3 hours. Table VI gives the infection experiments carried out with

TABLE V.

Infection Experiments with Patients' Blood Withdrawn before and after Intravenous Injection of 20 Cc. of Serum.

Positive Infections.

No of serum.	Day of illness.									
	3		4		5		6		7	
	Before injection.	After injection.	Before injection.	After injection.	Before injection.	After injection.	Before injection.	After injection.	Before injection.	After injection.
2			4	0	1	0	1	0	2	0
6	1	1	1	0	3	0	1	0	1	0
7	3	1	3	3	1	1	1	1		
8			1	1			1	0		

TABLE VI.

Infection Experiments with Blood Withdrawn after Serum Injections.

Time between serum injection and withdrawal of blood.	Infection experiments before serum injection.	Infection experiments after serum injection.
hrs.		
1–3	+ 19	− 13
		+ 6
Over 5	+ 5	− 4
		+ 1

blood withdrawn after serum injection. These results show that serum having a titer of 0.01 cc., given intravenously in doses of 20 cc., after 3 hours destroys completely spirochetes contained in the peripheral blood, which then is incapable of infecting guinea pigs. While with the use of sera having a titer of 0.03 cc., we found 3 hours after injection incomplete destruction of spirochetes in the blood,

the animals became typically ill later than the controls. It would appear, then, that the spirochetes had become reduced in number or had lost some of their virulence.

Behavior of the Immune Bodies in the Blood.

Experiments were conducted with the blood of twenty patients. Owing to a shortage of guinea pigs, we were not able to inject the animals immediately after withdrawal of the blood, and the fluid was placed in the refrigerator until it could be used. Unfortunately, most of these specimens became contaminated. The results of our investigation for the presence of immune bodies in the blood during the course of the disease, after intravenous injection of immune sera, are shown in Table VII.

The immune bodies appear in the blood much earlier with intravenous than with subcutaneous injections. They are present as

TABLE VII.

Appearance of Immune Bodies in the Serum of Intravenously Injected Patients.

Case No.	Day of illness when serum was injected.	Day of illness when blood was withdrawn.						
		6	7	8	9	10	11	12
8	4	−						÷
4	3		−		+			
2	3		−					
11	4	−		+				
10	4			+				
7	4			+				
6	4	±(+7)		+				
14	5				+			
38	5		+					
19	5		+					
15	5		±(+4)					+
23	6	−		+				
24	6	+		+				
21	6				+			
20	6			+				
30	7		−		+			
33	7						+	
28	7				+			
29	7				+			
34	8				+			

early as the 8th or 9th day, which does not, as a rule, occur when the patient has received no serum treatment. They may be observed after the introduction of 60 cc. of immune serum.

In one of the cases referred to the immune bodies appeared as early as the 6th day, before the administration of serum was begun. It is possible that in this instance an error has been made in computing the day of illness, inasmuch as we have never observed a similar result with other non-serum-treated cases.

Mortality.

The total mortality with the use of the intravenous injections was 23.7 per cent. Of forty-one patients under observation, twelve died. We have not included in our tabulations a patient who after recovery from the disease died on the 55th day from complications, a patient admitted to the clinic in moribund condition who died after 29 hours (numerous spirochetes could be demonstrated in the blood), and another, admitted when moribund, who died after 31 hours. The mortality of patients receiving no serum was 30.6 per cent. In the subcutaneously injected patients, the percentage was 17.3. This low mortality in the case of the subcutaneously injected patients is attributable to the fact that in this group, which entered before the 8th day of illness, only five patients were over 40 years of age, while in the group of intravenously injected patients, twelve were over that age.

Since in the moderate and slight forms of Weil's disease death results from complications, we shall consider particularly the severer cases of the disease. As the serum is effective only when administered in the initial stage, we have not considered in our study patients admitted after the 6th day. The mortality of patients who were admitted before the 7th day and received no serum was 57.1 per cent, of the subcutaneously injected patients 40 per cent, and of the intravenously injected patients 38.5 per cent.

It is clear that the total mortality figures, particularly the mortality of the severer cases, is considerably lower in the case of intravenous and subcutaneous serum administration than in the non-serum-treated cases.

In judging the results, one should take into account factors which strongly influence the prognosis. Among these factors must be counted the age of the patient, the severity of the illness, the season of its occurrence, and above all the day of illness on which serum was first administered. One must also consider whether the cases occur in epidemic form or sporadically. The difficulty of estimating the results is further increased by the fact that often at the onset it is not possible to judge precisely the degree of severity of the disease, while on the 6th or 8th day an error in prognosis is unusual. Table VIII shows the influence of the age of the patient upon the prognosis.

TABLE VIII.

Mortality and Age.

Age.	Without serum treatment.		Intravenous serum treatment.
	Inada.	Nagao.	
yrs.	*per cent*	*per cent*	*per cent*
Up to 20	12.5	60.0	0
21–30	21.0	17.9	12.5
31–40	23.5	20.0	0
41–50	21.4	35.0	42.8
51–60	75.0	50.0	33.3
Over 60	50.0	75.0	100.0

Of 21 patients less than 40 years old, admitted from the 3rd to the 7th day of illness, who were treated by intravenous injections of serum, only 1 died. This was a patient admitted on the 7th day. The 21 cases included 4 slightly ill, 7 moderately ill, and 10 severely ill patients. Of the 12 patients over 40 years of age—2 slightly ill and 10 severely ill—7 died. Prior to the age of 40, a good result is insured from the serum injections, but after 50 the outlook is not favorable; the older persons constitute, in fact, the severest cases of the disease. When we compare 10 severe cases below 40 years with 10 above that age, we find among the first only 1 death as against 7 deaths in the older group. It is evident that the years above 40 exert an unfavorable influence on the prognosis of Weil's disease, and this is especially true when the disease appears in severe form.

Effect of Intravenous Serotherapy on the Spirochetes in the Organism.

The spirochetolytic action of the immune serum upon the spirochetes in the human body is more pronounced with the intravenous than with the subcutaneous injection. Kaneko and Okuda have studied this phase of the subject.[2]

Effect of Serotherapy upon the Symptoms and Course of Weil's Disease.

Severity of Illness.—When the serum is administered early, the disease appears to assume a milder form. As already stated, it is difficult at the beginning to judge the precise degree of illness. We observed one patient moderately ill and another slightly ill, who after the serum injection could be grouped with the atypical cases. It is, however, not possible to assert that this change is wholly attributable to the serum; it may be that the disease in these cases would have run a less severe course without the administration of serum. But when we study the influence of the intravenous injection upon the different symptoms, we find that on the whole they are considerably diminished and shortened in duration.

Fever appears not to be influenced by the serum to any extent, even in cases where serum is administered early in the disease. In fact we often observed a rise in temperature after an injection, though it is not possible to say that this was due to the action of the serum.

The immune serum has a favorable effect on the duration of the icterus. The exact time of disappearance cannot be definitely stated on the basis of an examination made of the skin and mucous membranes, inasmuch as anemia usually follows recovery. We have, therefore, taken as an index Gmelin's urine test, which has been used in our clinic during the past 10 years. Without serum treatment, the icteric condition usually continues for a period of 20 days or more in the severe cases, sometimes as long as 30 days; and in the moderately ill 14 to 30 days, occasionally more than that time. In the cases receiving subcutaneous serum treatment, the icterus continued for an equal length of time. No definitely favorable effect was observed, even in the cases receiving an injection as early as the 3rd or 4th

[2] Their work is reported in detail on page 305 in this *Journal*.

day of illness. On the other hand, the icterus of the intravenously treated patients in most cases disappeared within 20 days, in the earliest case on the 13th day. No such finding was ever observed in the non-serum-treated cases or in those receiving subcutaneous injections. Of the severe cases receiving the intravenous injection prior to the 6th day, in only two did the icterus last more than 20 days. The longest period was 12 to 14 days in moderately severe cases. When the injection of serum is deferred until after the 6th or 7th day, the icteric condition will continue as long as it would if no serum had been given. We cannot state definitely whether an injection made on the 1st or 2nd day is capable of suppressing the icterus completely, but this is probable. Of the eight patients who received serum treatment on the 3rd or 4th day and at that time showed no signs of icterus, three remained without jaundice throughout. In any event we may say that the duration of the icterus is shortened considerably when the intravenous injections are given early in Weil's disease. The intensity of the icterus in most cases is greatly diminished by the intravenous injections (Tables IX, X, and XI).

The serum has likewise a decided influence upon hemorrhages. This was observed to a slight extent also with the subcutaneous injections. As a rule, cutaneous hemorrhages continue, but they are less pronounced in character. The percentage of petechiæ in the patients receiving intravenous treatment was 70.7, while in those receiving no serum it was 72.2. Although there is little difference in the figures, the duration of this symptom in the first group was considerably shorter. In half of the non-serum-treated cases the petechiæ continued for 10 days, while in the cases which received serum intravenously (admitted from the 1st to the 6th day of illness) only a third showed this symptom for more than 9 days. The number and size of the petechial spots were also reduced. Hemorrhage from the nose occurred also with serum treatment, but the percentage was somewhat smaller; that is, 29.3 against 32.7 per cent. Hemorrhages from the mucous membranes, gums, and tongue, and hematoma of the buccal mucosa continued to occur. In the intravenously treated cases the proportion was 41.5 per cent, in the non-serum-treated cases, 43.5 per cent. When the intravenous injection

was given early, within the first 6 days, the percentage was 30.6. This is true of intestinal hemorrhages, which may be reduced one-half by the intravenous injections. Table XII gives in percentages the

TABLE IX.

Duration of Icterus with Intravenous Serum Injection.

Day of illness when serum was injected.	Degree of illness.	Duration of icterus.
		days
3 (four cases).	Atypical.	Without icterus. Icterus only of conjunctiva bulbi.
	"	13
	Severe.	17
	"	
4 (five cases).	Atypical.	Without icterus.
	Severe.	15
	Moderately severe.	14
	Severe.	15
	"	23
5 (five cases).	Moderately severe.	Without icterus.
	Severe.	8
	Moderately severe.	12
	Severe.	16
	"	21
6 (six cases).	Atypical.	Without icterus. Icterus only of conjunctiva bulbi.
	Slight.	11
	Moderately severe.	12
	Slight.	13
	Moderately severe.	18
	Severe.	
7 (six cases).	Severe.	16
	Moderately severe.	14
	" "	16
	Severe.	17
	"	23
	"	30
8 (two cases).	Moderately severe.	16
	Severe.	28
9 (two cases).	Moderately severe.	13
	Severe.	23
Total 30		

hemorrhages occurring in the non-serum-treated cases, the intravenously treated cases, and of the latter those receiving serum injections at an early stage. The table also shows the percentages of hematemesis and epistaxis.

TABLE X.

Duration of Icterus with Subcutaneous Serum Injection.

Day of illness when serum was injected.	Degree of illness.	Duration of icterus.
		days
3 (one case).	Atypical.	4
4 (one case).	Severe.	22
5 (three cases).	Severe. Moderately severe. Severe.	16 20 24
6 (six cases).	Slight. Severe. Moderately severe (approaching slight form). Severe. Moderately severe. Severe.	14 18 20 20 29 33
7 (three cases).	Severe. " "	20 24 30
8 (five cases).	Slight. Moderately severe (approaching slight form). Moderately severe. " " " " (approaching severe form).	10 20 24 25 26
9 (one case).	Severe.	20
13 (one case).	Severe.	17 (died on 34th day).
Total 21		

From the above considerations we may conclude that the early intravenous injection of immune serum has a decidedly favorable influence upon the hemorrhagic tendency of Weil's disease, and particularly upon hemorrhages from the mucous membranes.

TABLE XI.

Duration of Icterus without Serum Treatment in Thirty-Six Cases.

Degree of illness.	Duration of icterus.
	days
Atypical.	Icterus only of conjunctiva bulbi.
"	5
Slight.	11
"	12
Moderately severe.	14
" "	14
" "	17
Slight.	17
Moderately severe.	18 (died on 18th day).
Severe.	20
"	20
Moderately severe.	21
Slight.	22
Moderately severe.	22
Severe.	24
"	24
"	24
"	25
"	25
Moderately severe.	22
" "	25
" " (approaching severe form).	25
" "	26
" "	28 (had beri-beri).
" "	29
" "	30
" " (approaching severe form).	30
Severe.	30
"	31
"	34
"	37+
Moderately severe.	37+
" "	38+
" "	24+
Severe.	24+
"	29+ (died on 38th day).

TABLE XII.

Percentage of Hemorrhages in the Serum-Treated and Non-Serum-Treated Cases.

Symptoms.	Without serum treatment.	Intravenous serum treatment.	Intravenous serum treatment (26 cases admitted from 3–6 days).
	per cent	*per cent*	*per cent*
Hemorrhages from tongue and mouth	43.5	41.5	30.6
Intestinal hemorrhages	30.2	22.0	15.3
Hematemesis	11.3	9.7	7.6
Bloody stools	15.1	7.3	7.6
Petechiæ	72.2	70.7	69.2
Epistaxis	32.7	29.3	
Complications—suppurative processes	24.0	14.6	4.9

We have not observed a decidedly favorable influence of the serum upon the pulse, although arrhythmia was a less frequent symptom. It occurred in 20 per cent of the cases, while in the non-serum-treated cases the figure was 50 per cent.

Complications such as suppurative processes—parotitis, skin abscesses—were found in 14.6 per cent, in patients admitted from the 3rd to the 6th day only in 4.9 per cent, and in patients receiving no serum treatment in 24 per cent of the cases. Thus these complications are greatly reduced with the early use of the serum.

Summarizing the observations on such symptoms as hemorrhage, particularly of the mucous membranes, heart rhythm, and suppurative processes, which play a large part in the outcome of the disease, we are justified in saying that the intravenous injection of immune serum has a definitely beneficial effect.

The after-fever was found to occur somewhat more frequently—in 34.1 per cent—while without serum treatment it occurred in only 28.2 per cent. The greater frequency of this symptom is probably referable to the fact that the most severely ill patients who recover from the disease are included in this category. As the time of greatest mortality of Weil's disease lies between the 8th and the 18th days, and the after-fever, as a rule, does not begin until the 13th or 15th day, it is evident that this symptom does not usually occur in the cases ending fatally. The percentage of after-fever is found to increase as the mortality decreases.

On two occasions following the injection of Serum 7, and once after Serum 2, the patients had chills followed by a rise in temperature. Serum rash occurred three times after the administration of Serum 2. There were no anaphylactic manifestations.

Among 18 patients who returned for examination after several months we found 8 who showed more or less marked ocular disturbances. These 18 cases comprised 10 severe, 5 moderately severe, and 3 atypical forms of the disease. The 8 patients showing ocular disturbances were as follows: 5 from the severely ill group, 1 from the moderately ill, and 2 from the atypical group. 1 patient showed, on examination in the ophthalmologic clinic, iridocyclitis, hypopyon of the right eye, and opacity of the vitreous humor; in another only the last symptom was present. These after-effects seem to occur more frequently in the severe types of the disease. We cannot say at this time to what extent the sequelæ are influenced by serum treatment.

DISTRIBUTION OF SPIROCHÆTA ICTEROHÆMORRHAGIÆ IN THE ORGANS AFTER INTRAVENOUS SERUM TREATMENT.

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The material here reported was obtained from eight cases of Weil's disease in which postmortem examinations were made. The patients had all received intravenous serum treatment before the 8th day of illness. The distribution of spirochetes within the organs differs somewhat in these patients from that in those receiving no serum treatment. The latter have already been described by us.[1] In Table I are given our findings on the density of the spirochetes in the various organs, and also the findings in four non-serum-treated cases showing no complications.

It will be observed that in the serum-treated cases the spirochetes are more sparsely distributed than in the other group. In the former we were able to demonstrate microorganisms constantly in the kidneys, often in the heart muscle and the appendix, but only once in the liver, suprarenals, and lymph glands, and in three cases in muscle tissue. The spirochetes were few in number and the specimens observed were markedly degenerated. With the exception of one case, but few spirochetes could be discovered in the liver, while in the non-serum-treated cases in this stage of the disease there was no difficulty in demonstrating spirochetes in that organ, although they were not numerous. In a patient dying on the 6th day, who had received no serum treatment, we found a dense distribution of spirochetes in the liver. The pancreas, with the exception of one case, and the spleen, lungs, and other organs of the serum-treated patients showed no spirochetes.

[1] Kaneko, R., and Okuda, K., *J. Exp. Med.*, 1917, xxvi, 325.

TABLE I.

Spirochetes Found in the Organs in Weil's Disease.

Serum-Treated Cases.

Case No.	Age.	Sex.	Day of illness when serum was given.	Amount of serum.	Day of death.	Liver.		Kidneys.		Supra-renals.	Spleen.	Lymph glands.	Appendix and intestine.	Pancreas.	Lungs.	Heart.	Bladder.	Prostate.	Muscle.
						Extra-cellular.	Intra-cellular.	Interstices.	Tubules.										
	yrs.			*cc.*															
1	30	Male.	5	20	5	−	−	+	−	−	−	−	+	−	−	+		−	−
2	23	"	5	20	5	−	−	+	+	−	−	−	+	+	−	−	+	+	+
3	57	"	4	20	6	−	−	+	+	−	−		+	−		+	+	−	+
			5	20															
4	56	Female.	6	20	8	−	−	+	+	+	−	−	−	−	−	+	−		−
			7	20															
5	50	Male.	5	20	9	−	−	+	+	−	−	+	−	−	−	+		−	−
			6	20															
			7	20															
6	30	"	7	20	11	−	−	+	+		−	−	−	−	−	−			+
			8–10	40															
7	47	"	3	20	11	−	−	−	+	−	−	−	+	−	−	−		−	−
			4	20															
			5–6	20															
8	73	Female.	4	20	11	−	+	+	+		−	−	+		−	−			−
			5	20															

Non-Serum-Treated Cases.

9	59	Male.			6	+++	+	++	+	++	+			+	+				
10	45	"			7	+	+	++	+		+	+	+	++	+	++	+	++	+
11	28	"			8	−	+	+++	+	+	+	+	+++	+	−	++	++	++	+
12	14	Female.			10	+	+	+++	+	+	+	+	+	+	+	+	+		+

+, very few spirochetes—one or a few in a preparation.

+, spirochetes sparsely distributed. The spirochetes can be found readily, more than 10 to 20 in a preparation, or one in several fields.

++, relatively numerous spirochetes, one or a few in a single or several fields, with numerous spirochetes in a preparation.

+++, numerous spirochetes, some being found in almost every field, and often numerous specimens in a single field.

−, negative finding in one or more preparations.

CONCLUSIONS.

On the basis of these findings we believe that we are justified in saying that the immune serum of Weil's disease is capable of destroying the spirochetes found within the organs in man, with the exception of the kidneys, and that the action of the serum upon the spirochetes is spirochetolytic and spirocheticidal. The scattered spirochetes in the kidney, on the other hand, are resistant to the action of the immune serum.

The spirochetes disappear almost completely from the organs during the convalescent stage of Weil's disease, even when no serum has been administered. The only organ to be excepted is again the kidney, but no comparison between serum-treated and non-serum-treated cases should be made in this respect, for spirochetes are found numerously in the kidneys even with serum treatment.

The disappearance of the spirochetes from the organs and tissues in Weil's disease seems to be not so marked with the subcutaneous serum treatment as with the intravenous method, but the manner of their disappearance is about the same.

We desire to express to Professor R. Inada, our appreciation of his interest in the work.

THERAPEUTIC EXPERIMENTS WITH ROSENOW'S ANTI-POLIOMYELITIC SERUM.

By HAROLD L. AMOSS, M.D., and FREDERICK EBERSON.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

Plates 8 to 10.

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Investigators are divided into two well defined groups with reference to the interpretation of the relation of certain streptococci cultivated from poliomyelitic nervous tissues to epidemic poliomyelitis. One group[1] affirms that the streptococci bear a causal relation to poliomyelitis and are even related biologically to the globoid bodies of Flexner and Noguchi,[2] while another group[3] denies that they possess any essential etiologic importance and views them merely as secondary invaders.[4]

The question at issue is an important one in every way, because upon its true answer will depend the prophylactic measures adopted to prevent epidemics of poliomyelitis and the direction which effort will take in perfecting an efficient agent for specific therapy.

Until recently the effort made to treat cases of poliomyelitis specifically has been with the blood serum of convalescent and recovered cases of the disease. This procedure is based upon several kinds of conclusive experimental data. However, too few observations are available to decide whether the method gives unmistakable thera-

[1] Mathers, G., *J. Am. Med. Assn.*, 1916, lxvii, 1019; *J. Infect. Dis.*, 1917, xx, 113. Rosenow, E. C., Towne, E. B., and Wheeler, G. W., *J. Am. Med. Assn.*, 1916, lxvii, 1202; *Science*, 1916, xliv, 614; *J. Am. Med. Assn.*, 1917, lxviii, 280. Nuzum, J. W., and Herzog, M., *J. Am. Med. Assn.*, 1916, lxvii, 1205. Nuzum, J. W., *ibid.*, 1916, lxvii, 1437; 1917, lxviii, 24.

[2] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

[3] Bull, C. G., *J. Exp. Med.*, 1917, xxv, 557. Kolmer, J. A., Brown, C. P., and Freese, A. M., *J. Exp. Med.*, 1917, xxv, 789.

[4] Smillie, W. G., *J. Exp. Med.*, 1918, xxvii (in press).

peutic results in human cases of poliomyelitis, although the indications are favorable.[5]

The employment of the serum derived from recovered cases of poliomyelitis followed not only on account of the detection of its neutralizing property *in vitro* for the poliomyelitic virus, but also because of the failure to induce antibody formation in a variety of domestic animals, including the horse, by the injection of the nervous tissues of monkey or man carrying the virus.[6] Incidentally, it may be stated that only imperfect success in developing antibodies in rabbits and monkeys has attended the repeated injection of cultures of the globoid bodies.[7]

A far greater measure of success has been claimed for the streptococci in producing antibodies for the virus of poliomyelitis. Rosenow[8] and Nuzum and Willy[9] assert that animals immunized with the streptococci cultivated from poliomyelitic cases exhibit various antagonisms to the virus. Monkeys inoculated with streptococci are said to be protected from subsequent infection with the poliomyelitic virus; the blood of the protected monkeys is stated to be neutralizing *in vitro* for the virus; and finally, horses immunized with the virus are said to yield a serum which possesses neutralizing, protective, and therapeutic properties, even when applied to man.

It is this last statement which calls for painstaking control. It is obvious that to obtain a decision from the treatment of human cases of poliomyelitis would require a large and varied series of observations extending over a long period of time and embracing epidemics of considerable magnitude and various degrees of severity. Moreover, the observations would have to be carefully controlled by comparison with an equal number of cases, occurring simultaneously, untreated with the antiserum, under approximately identical conditions and equally accurately studied. To secure these data might require several years, as has been the case notably with the serum treatment of diphtheria and epidemic meningitis. The question arises,

[5] Amoss, H. L., and Chesney, A. M., *J. Exp. Med.*, 1917, xxv, 581.
[6] Flexner, S., *J. Am. Med. Assn.*, 1910, lv, 1105.
[7] Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 545.
[8] Rosenow, E. C., *J. Am. Med. Assn.*, 1917, lxix, 261, 1074.
[9] Nuzum, J. W., and Willy, R. G., *J. Am. Med. Assn.*, 1917, lxix, 1247.

therefore, whether a method is not available by means of which a probable decision may be reached more expeditiously and with greater certainty. It is because of our belief that a decisive experimental method is at hand that the series of experiments to be reported were performed.

EXPERIMENTAL.

The injection intracerebrally into monkeys of minute quantities of an active virus of poliomyelitis is followed by paralysis and, as a rule, by death of the animal. The injection of far greater quantities of the same virus into the blood stream produces no symptoms. If, however, as Flexner and Amoss[10,11] have shown, the meninges and choroid plexus are chemically inflamed by a simultaneous or previous injection of sterile horse serum, monkey serum, or even isotonic saline solution, the virus is enabled to pass from the blood into the nervous tissues and thus to induce the characteristic changes which lead to paralysis and even to death. The same authors found only one substance which, when injected intraspinally, prevented the localization of the virus in the nervous organs after intravenous injection, and that is the serum derived from monkeys which have survived a poliomyelitic infection. Moreover, this serum is capable of setting aside the effects of the chemical inflammation incited by horse serum or other foreign fluids injected intraspinally. In other words, when the immune convalescent serum is introduced into the meninges in animals previously injected intraspinally with horse serum or other fluids mentioned, followed by an intravenous injection of the virus, no paralysis or other evidence of infection results. This experiment gives such decisive and unequivocal results that it seems particularly adapted to determine the therapeutic value of a serum or other product reputed to be effective in the treatment of poliomyelitis in man.

The experiments to be described were carried out in the following manner. An active, fresh poliomyelitic virus was obtained in the usual manner by inoculating a monkey intracerebrally with a suspension of glycerolated virus. On the 1st day of complete prostration, the animal was etherized, and the brain and spinal cord were

[10] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.
[11] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 525.

aseptically removed. With the spinal cord and medulla, a 5 per cent suspension in isotonic salt solution was prepared, shaken, and centrifuged, and the clear supernatant fluid injected intravenously into *Macacus rhesus* monkeys. Two separate sets of experiments were performed. Control animals and animals treated with Rosenow's serum, with normal horse serum, and with convalescent monkey serum, in the same manner, were tested simultaneously. The protocols follow. The outcome is as sharp and hence as decisive as the experimental results could make it.

Experiment 1.—Monkey A, control. Nov. 13, 1917. Injected intravenously 50 cc. of the virus prepared as described. The animal remained well.

Monkey B, normal horse serum control. Nov. 12, 1917, 5.10 p.m. Injected intraspinally 2.5 cc. of normal horse serum. Nov. 13, 11.15 a.m. Injected intravenously 50 cc. of virus. 12 noon. Injected intraspinally 2.5 cc. of normal horse serum. Nov. 14. Injected intraspinally 2.5 cc. of normal horse serum. Nov. 15. Repeated intraspinal injection of normal horse serum. Nov. 16. Repeated intraspinal injection of normal horse serum. Protects left leg; ataxic. Nov. 17, a.m. Left arm, right deltoid, and both legs paralyzed; head tremor; ptosis of the left eyelid; almost prostrate. 4.30 p.m. Died.

Autopsy.—Macroscopic and microscopic lesions of poliomyelitis. No visible changes in viscera.

Monkey C, Rosenow's serum. Nov. 12, 1917, 4.40 p.m. Intraspinal injection of 3 cc. of Rosenow's antipoliomyelitic horse serum activated with one-ninth volume of fresh guinea pig serum. Nov. 13, 10.45 a.m. Intravenous injection of 50 cc. of virus. 10.50 a.m. Intraspinal injection of 3 cc. of Rosenow's serum. Nov. 14, 15, 18, 19, and 20. Injected 3 cc. of Rosenow's serum, intraspinally. The clinical course of the animal was as follows: Nov. 17. Slow. Nov. 19. Excited; slow; protects right leg. Nov. 20. Drags right leg; climbs awkwardly. Nov. 22. Both legs weak. Nov. 23. Legs paralyzed; deltoids weak. Nov. 24. Prostrate (Fig. 1). Nov. 26. Moribund; etherized.

Autopsy.—The spinal cord and brain were edematous and the gray matter was congested. Microscopic examination of the central nervous system showed marked perivascular infiltration and some neurophagocytosis in the gray matter of the medulla and cervical enlargement characteristic of poliomyelitis. Perivascular infiltration (Figs. 2 and 3), congestion, neurophagocytosis, and meningeal infiltration in lumbar enlargement. Focal infiltration of lymphocytes, cell degeneration, and neurophagocytosis in the posterior root ganglia.

Monkey D, serum of recovered monkeys. Nov. 12, 1917, 4.55 p.m. Intraspinal injection of 3 cc. of mixed serums from several *rhesus* monkeys which had recovered from experimental poliomyelitis and subsequently received subcutaneous injections of the virus contained in the spinal cord and medulla (reinforced

immune). Nov. 13, 11 a.m. Intravenous injection of 50 cc. of virus suspension followed by intraspinal injection of 3 cc. of immune serum. Nov. 14, 15, 18, 19, and 20. Intraspinal injections of 3 cc. of immune serum. The clinical course of this animal was in striking contrast with the preceding. At no time were any symptoms present; the animal continued apparently normal throughout the treatment and is well at the present time (Jan. 1, 1918).

This experiment was repeated with precisely the same results. The protocols follow. In the second experiment, the normal horse serum control was omitted.

Experiment 2.—Monkey E, control. Nov. 26, 1917. Intravenous injection of 50 cc. of the virus. No symptoms appeared, and the animal has remained normal up to the present time (Jan. 1, 1918).

Monkey F, Rosenow's serum. Nov. 26, 1917, 6 p.m. Intraspinal injection of 2 cc. of activated Rosenow's antipoliomyelitic horse serum. Nov. 27, 11.35 a.m. Intravenous injection of 50 cc. of virus, followed immediately by the intraspinal injection of 2.5 cc. of Rosenow's serum. The intraspinal injections were repeated on Nov. 28, 29, Dec. 2, and 3. In each instance the activated serum was injected. Dec. 3. The animal developed a marked tremor of the head, ataxia, and right facial paralysis; also, the deltoid muscles were weak. Dec. 4. The monkey died in the early morning.

Autopsy.—Macroscopic lesions of poliomyelitis throughout brain and cord. Microscopic examination of the central nervous system showed marked congestion and perivascular infiltration, slight cell degeneration, and neurophagocytosis in medulla and cervical enlargement (Fig. 4); slight meningeal infiltration in lumbar enlargement, and focal infiltration of lymphocytes, cell degeneration, and neurophagocytosis in posterior root ganglia (Fig. 5).

Monkey G, immune monkey serum. Nov. 26, 1917, 5.15 p.m. 2 cc. of pooled immune serum injected intraspinally. Nov. 27, 12.25 p.m. Intravenous injection of 50 cc. of the virus, followed by the intraspinal injection of 2.5 cc. of pooled immune serum. The intraspinal injections of the pooled serum were repeated on Nov. 28, 29, Dec. 2, and 3. At no time were any symptoms detected, and the animal is normal at this time (Jan. 1, 1918).

DISCUSSION.

The preceding experiments accomplish two purposes directly. First, they test the ability of Rosenow's serum, which was prepared by injecting a horse with cultures of the streptococci derived from poliomyelitic nervous organs, to prevent a poliomyelitic infection arising in the monkey after an intravenous injection of the virus. This is readily accomplished by means of the immune serum obtained

from convalescent and recovered monkeys. Second, they compare directly under these favorable therapeutic conditions the Rosenow serum with the serum of immune monkeys.

The results of the experiments are unequivocal. They show the Rosenow serum to be devoid of protective power. Moreover, they show that the Rosenow serum acts in the manner of normal horse serum in promoting infection in monkeys from an intravenous injection of the virus, in itself incapable of inducing paralysis.

The immune monkey serum possesses, under the same conditions of administration, perfect protective power, as has been shown previously by Flexner and Amoss.[11]

A further conclusion may be drawn from the experiments. Rosenow states that the horse serum prepared by him contains demonstrable antibodies for the streptococci employed in its production. It is assumed that these antibodies are identical with the antibodies, demonstrable by neutralization experiments with the virus, contained within human and monkey serum derived from recovered cases of poliomyelitis in man and the monkey. This supposition is rendered untenable by the results of our experiments. The antibodies induced in the horse by immunization with the streptococci have proven incapable of neutralizing the virus of poliomyelitis introduced into the blood of monkeys in its passage to the central nervous system—a neutralization which the immune monkey serum readily effects. The two classes of antibodies or immunity principles, those present in the blood derived from recovered cases of poliomyelitis and those induced in the horse by treatment with streptococci, are therefore to be regarded as distinct.

There is a further corollary to this general deduction. Once it is established that the antibodies yielded by the streptococci differ essentially from those induced by the virus of poliomyelitis, the contention that virus and streptococci are identical becomes untenable. In other words, the experiments reported in this paper tend also to refute the claim that certain streptococci are the microbic cause of epidemic poliomyelitis.

Rosenow and Nuzum and Willy assert that their serums possess striking therapeutic activity in man. Their conclusions are based on the treatment of relatively small numbers of cases of epidemic polio-

myelitis during the past summer and autumn. We have already drawn attention to the difficulties surrounding a statistical study, of limited extent, of the questions here involved. Hence we venture to place, in this instance, the greater weight on decisive animal experiments, and those reported in this paper clearly show that Rosenow's horse serum injected intraspinally into monkeys is without specific protective power against the virus of poliomyelitis.

It has, however, been shown by Flexner and Amoss[12] that normal horse serum, when injected intraspinally into monkeys, promotes the passage of poliomyelitic immune bodies from the blood into the subarachnoid space. Hence it is possible that under certain circumstances in which those bodies are already present in the blood in man, they may be directed into the subarachnoid space through the increased permeability of the meninges induced by the horse serum and thus affect the course of the infection. The antibodies have been detected on the 3rd[13] and 6th[12] days of illness in man, or, in other words, early in the course of the disease.

This, however, is a purely hypothetical consideration, in support of which normal horse serum should prove as effective as antistreptococcus serum. It is questionable whether this roundabout method of directing the circulating immunity bodies to the central nervous organs is advisable in practice. As far as present knowledge, based on definitive experiments, is concerned, it may be said that only immune serum derived from convalescent and recovered cases of poliomyelitis in man and the monkey have been determined to be protective against the infectious power of the poliomyelitic virus.

CONCLUSIONS.

Two series of experiments are described in which Rosenow's antipoliomyelitic serum, so called, has been compared with the immune serum derived from monkeys which have convalesced or recovered from experimental poliomyelitis.

The experiments consisted in introducing an active virus of polio-

[12] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 499.

[13] Kling, C. A., and Levaditi, C., Études sur la poliomyélite aiguë épidémique, Paris, 1913, p. 114.

myelitis into the blood and of injecting the two kinds of serum into the cerebrospinal meninges according to the method of Flexner and Amoss.

Under the conditions of the experiment, the control monkeys (*a*) receiving the virus intravenously alone do not develop paralysis, while those (*b*) receiving the virus intravenously and normal horse serum intraspinally develop paralysis. Moreover, the monkeys (*c*) receiving the virus intravenously and Rosenow's antipoliomyelitic serum intraspinally develop paralysis in the manner of those receiving normal horse serum intraspinally. The monkeys (*d*) which received the virus intravenously and the convalescent or immune monkey serum intraspinally alone did not develop paralysis.

The Rosenow serum acts in the manner of normal horse serum; it promotes the passage of the virus of poliomyelitis from the blood into the nervous organs, and it does not protect from infection.

We have found no evidence that Rosenow's serum under the conditions of the tests is effective therapeutically in monkeys or possesses antibodies of the same nature as those present in the blood of monkeys which have recovered from experimental poliomyelitis.

Since the antibodies in convalescent poliomyelitic serum in man and the monkey are identical, it follows that any antibodies present in the Rosenow horse serum do not conform to those occurring in human convalescent serum.

EXPLANATION OF PLATES.

The illustrations were taken from monkeys treated with Rosenow's serum.

PLATE 8.

FIG. 1. Monkey C. 11 days after the intravenous injection of virus. Received seven intraspinal injections of Rosenow's serum. Arms, legs, and back muscles paralyzed; face muscles active.

PLATE 9.

FIG. 2. Monkey C. Cervical enlargement showing perivascular mononuclear cell infiltration in anterior horn. × 165.

FIG. 3. Monkey C. Cervical enlargement showing perivascular mononuclear cell infiltration in posterior horn. × 165.

PLATE 10.

FIG. 4. Monkey F. Cervical enlargement showing anterior horn with degeneration of ganglion cells and neurophagocytosis. × 240.

FIG. 5. Monkey F. Posterior root ganglion with ganglion cell degeneration, neurophagocytosis, and mononuclear cell infiltration. × 240.

FIG. 1.

(Amoss and Eberson: Rosenow's antipoliomyelitic serum.)

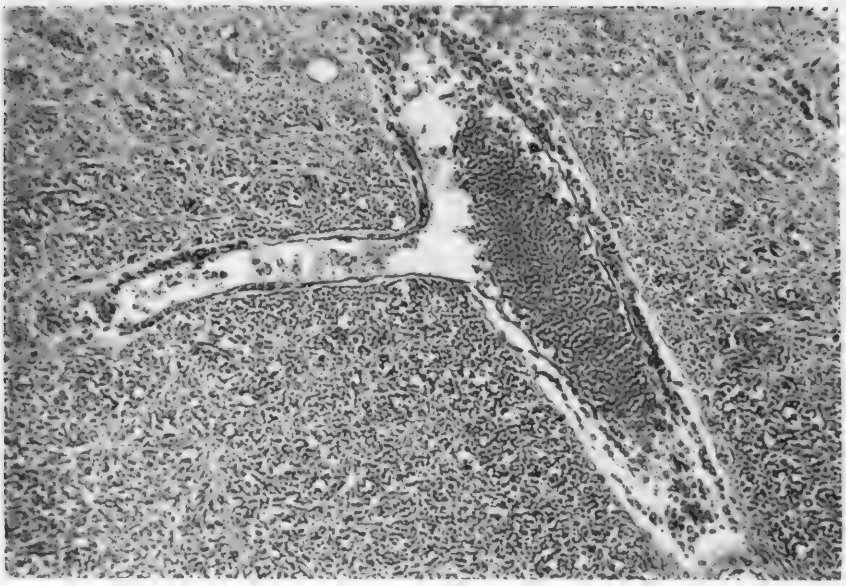

FIG. 2.

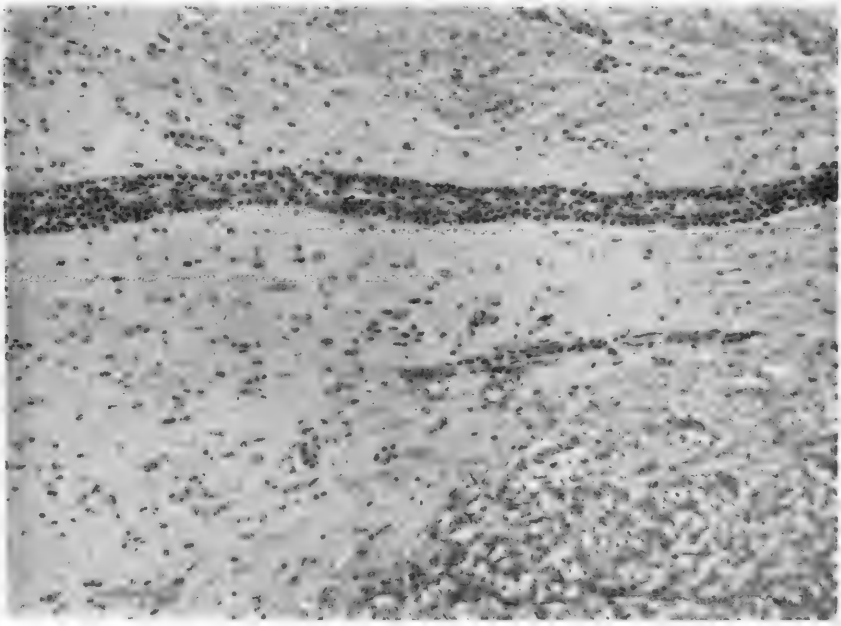

FIG. 3.

(Amoss and Eberson: Rosenow's antipoliomyelitic serum.)

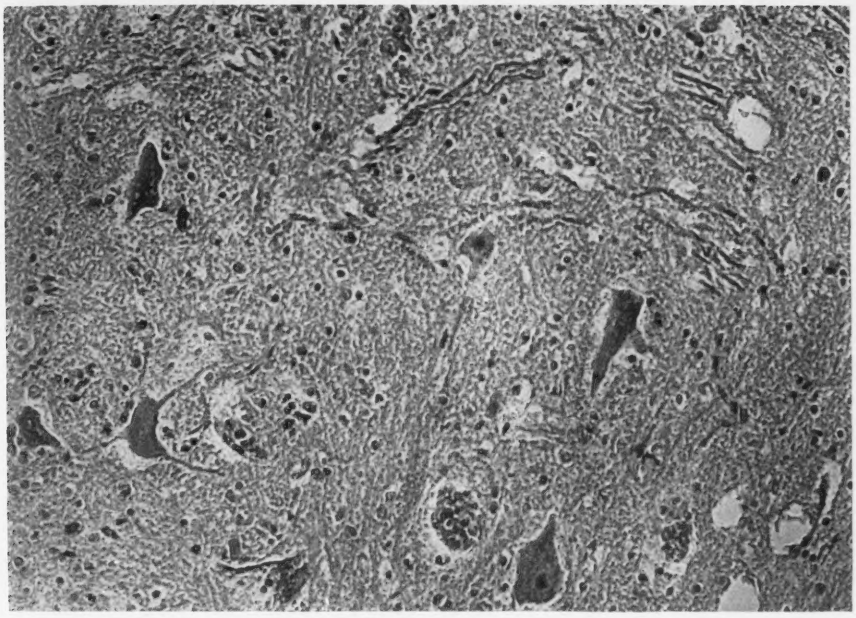

FIG. 4.

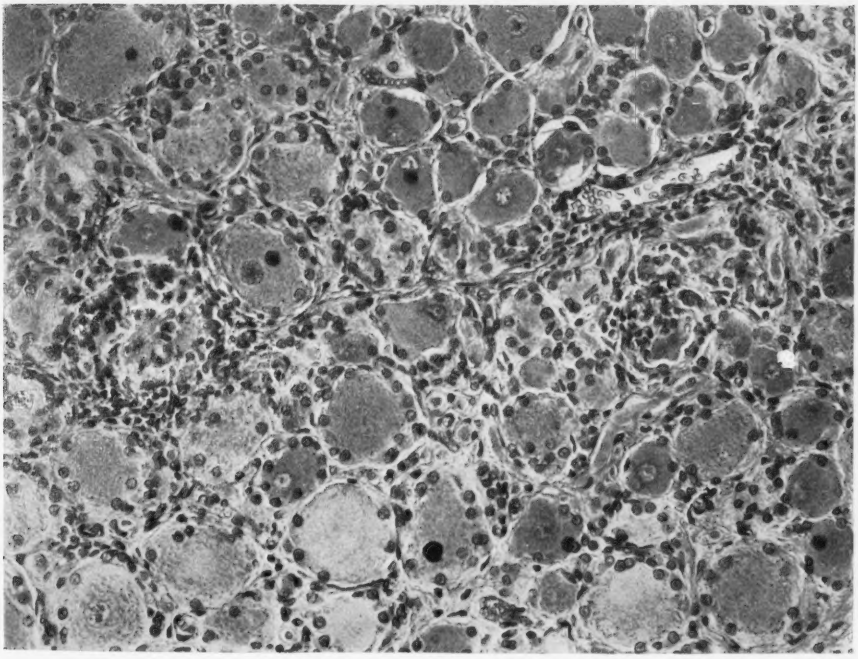

FIG. 5.

(Amoss and Eberson: Rosenow's antipoliomyelitic serum.)